Dissecting the Genetics of Major Depressive Disorder

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Abstract

Major depressive disorder (MDD) is a leading cause of disability worldwide and a major contributor to early mortality from suicide. It is a common psychiatric illness with a well-established heritability. MDD is an extremely heterogeneous disorder in terms of symptoms, genetics and environmental risk factors. This thesis uses genetic and environmental data from case-control, clinical trial and population samples to dissect the heterogeneity of MDD.

Gene-environment interactions were tested in the Radiant UK recurrent depression sample using polygenic risk scores (PRS), which reflect genetic liability for MDD based on many common variants. No interactions were found between PRS and adult adverse events. Interactions between PRS and childhood trauma showed an inverse association with MDD status, as cases who experienced more severe trauma tended to have a lower PRS.

The current selection pressures on genetic variants associated with MDD and other psychiatric disorders were investigated in the Icelandic population by testing whether PRS are associated with number of offspring. There was no evidence that risk alleles for depression are under selection, whereas higher PRS for autism were associated with fewer children and higher PRS for ADHD were associated with having more children.

Genome-wide association studies on suicide attempt were conducted comparing 6,569 attempters versus 17,232 non-attempters from MDD, bipolar disorder and schizophrenia cases in the Psychiatric Genomics Consortium. This identified three genome-wide significant loci for suicide attempt in mood disorders, which will be replicated in independent samples. Finally, blood mRNA levels of *SAT1, PTEN, MAP3K3 and MARCKS* have been reported as biomarkers for suicidality and here, an independent test in the Genome-Based Therapeutic Drugs for Depression (GENDEP) study did not support the validity of the proposed biomarkers. The availability of phenotypic, genetic and environmental data provides abundant opportunities to leverage the heterogeneity of depression to better understand its complex aetiology.

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Statement of authorship

Chapter 2: This invited review was written by Niamh Mullins and edited by Prof. Cathryn Lewis.

Chapter 3: The Radiant UK genotype data previously underwent quality control. The meta-analysis of PGC major depressive disorder cohorts excluding Radiant UK was conducted by Dr. Jack Euesden and polygenic risk scores for major depressive disorder had previously been generated by Dr. Ken Hanscombe. All statistical analysis was performed by Niamh Mullins. The first draft of the paper was written by Niamh Mullins and the paper was circulated amongst co-authors and underwent peer review prior to publication, leading to editing of the manuscript and suggestions for additional analyses.

Chapter 4: Genetic data had previously undergone genotyping, imputation, quality control and CNV calling. Polygenic risk scores were generated centrally by the statistics department at deCODE genetics. All statistical analyses were conducted by Niamh Mullins at deCODE genetics. During the peer review process, analyses were repeated by Dr. Andrés Ingason at deCODE genetics, using scripts written by Niamh Mullins, to include improved polygenic risk scores and additional CNVs. The first draft of the paper was written by Niamh Mullins and the paper was circulated amongst all co-authors prior to publication, leading to editing of the manuscript and suggestions for additional analyses.

Chapter 5: All cohorts were genotyped following their local protocols, after which standardised quality control and imputation were performed centrally using the Psychiatric Genomics Consortium 'Ricopili' pipeline. All statistical analyses were conducted by Niamh Mullins. The paper was written by Niamh Mullins and edited with feedback from Prof. Cathryn Lewis.

Chapter 6: Quality control on GENDEP gene expression data was performed by Dr. Karen Hodgson. Composite suicidal ideation scores had been generated previously by Dr. Nader Perroud. All statistical analysis was conducted by Niamh Mullins. The first draft of the paper was written by Niamh Mullins and the paper was circulated amongst co-authors and underwent peer review prior to publication.

1. Introduction

1.1 Major depressive disorder

Major depressive disorder (MDD) is a common psychiatric illness and a global public health problem. It is the third leading cause of years lived with disability worldwide and a major contributor to early mortality from suicide (GBD 2015 Disease and Injury Incidence and Prevalence Collaborators, 2016, World Health Organization, 2014). MDD is also associated with increased risk of developing comorbid physical disorders such as heart disease, diabetes and obesity and is predictive of poor outcomes for individuals with these conditions (Whooley and Wong, 2013). The burden of disease of depression goes beyond the impaired quality of life for those affected, to the substantial economic burden due to healthcare and work loss-related costs (Gustavsson et al., 2011).

MDD is defined as a two week period with either depressed mood or anhedonia and at least four other symptoms which may include weight and sleep changes, cognitive impairments, psychomotor symptoms and suicidality (American Psychiatric Association, 2013). Table 1 shows the nine core symptoms of MDD according to the current criteria from the Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-5) (American Psychiatric Association, 2013). These symptoms should represent a change from previous functioning and not be attributable to the effects of a substance, another medical condition or psychiatric disorder. The symptoms should cause clinically significant distress or impairment in social, occupational or other important areas of functioning (American Psychiatric Association, 2013).

Table 1: Nine core symptoms of MDD according to DSM-5 criteria

- Depressed mood*
- Markedly diminished interest or pleasure in all, or almost all, activities*
- Considerable weight loss when not dieting, weight gain, or decrease or increase in appetite
- Insomnia or hypersomnia
- Psychomotor agitation or retardation
- Fatigue or loss of energy
- Feelings of worthlessness, or excessive or inappropriate guilt, which might be delusional; that is, not merely self-reproach or guilt about being sick
- Diminished ability to think or concentrate, or indecisiveness
- Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan; the individual has made a suicide attempt or a specific plan for committing suicide

*One of these symptoms must be present for diagnosis

There is huge heterogeneity in the combination of symptoms which can lead to a diagnosis of MDD and the severity of MDD symptoms can vary widely (Ostergaard et al., 2011). However, the clinical course of the illness is often chronic or recurrent. In individuals receiving outpatient treatment, a quarter remit within six months and more than half experience a persistent episode lasting longer than two years (Otte et al., 2016). The majority of patients with MDD experience at least one recurrence in their lifetime (Judd, 1997). The lifetime prevalence of the disorder is approximately 15%, although this differs considerably across countries, with prevalence estimates generally higher in high income versus low-middle income countries (Weissman et al., 1996, Kessler and Bromet, 2013). Epidemiological studies consistently show that MDD is twice as common among women than men (Hasin et al., 2005, Kessler and Bromet, 2013). In both sexes, the median age at onset is around 25 years, with the peak risk period ranging from mid-late adolescence to the early 40s (Kessler and Bromet, 2013).

Treatment options for MDD are mainly pharmacotherapy, psychological therapy or a combination of both (Otte et al., 2016). Medication is typically prescribed for moderate to severe depression and most classes of antidepressant drugs act to increase the signalling of monoamine neurotransmitters, such as serotonin and noradrenaline. While antidepressants are effective for some patients, they have only modest efficacy, with improvement in symptoms taking several weeks and potentially adverse side effects such as sleep disturbances, headaches and weight gain (Otte et al., 2016, Undurraga and Baldessarini, 2012). There is a need for improved treatments for depression, however novel drug development has been hampered by limited understanding of the disorder's pathophysiology.

The aetiology of MDD is complex and multifactorial. There are a range of environmental risk factors for depression including social isolation, relationship stressors, financial problems and bereavement (Kendler et al., 1999). Childhood abuse or neglect is one of the strongest environmental risk factors, more than doubling the risk for depression in adulthood (Mandelli et al., 2015, Li et al., 2016). It has long been observed that depression aggregates in families. The risk of recurrence in the first degree relatives of an affected proband is 2.84 (Sullivan et al., 2000). Meta-analysis of concordance rates for MDD between monozygotic versus dizygotic twins has estimated the heritability of the disorder to be 37%, with unique environmental influences accounting for 63% of the variance (Sullivan et al., 2000). However, despite robust evidence for the heritability of MDD, identifying the genetic variants involved has been a major challenge. Over the last decades, advances in molecular genetic technology, computation and reduction in costs have revolutionised the field of genetics and allowed the study of genome-wide genetic data on hundreds of thousands of people. The next sections outline three statistical

genetics methods which have been successfully used to uncover the genetic architecture and specific genetic variants associated with complex diseases, including psychiatric disorders.

1.2 Genome-wide association studies

Genome-wide association studies (GWAS) test for differences in allele frequencies between disease and control groups at millions of biallelic single nucleotide polymorphisms (SNPs) across the genome. Common SNPs (minor allele frequency of at least 1%) are genotyped on microarrays and other SNPs in linkage disequilibrium (LD) with these variants can be imputed based on the correlation between them, obtained from sequenced reference samples. SNPs are tested individually for association with disease status using logistic regression and covarying for ancestry informative principal components, which control for subtle differences in population stratification between cases and controls, even within ethnically homogenous groups. Multiple testing correction is applied for one million statistical tests, which is the estimated number of independent common SNPs in the genome for European populations (Pe'er et al., 2008). In this way, GWAS take a hypothesis-free systematic approach, with no prior assumptions about which variants are likely to be involved in the disease. SNPs significantly associated with disease may be functionally relevant or may represent loci which are transmitted in LD with a causative polymorphism. The power of a GWAS is a function of the frequency and effect sizes of the alleles tested, sample size and the specified significance level. The stringent genome-wide significance threshold of $P < 5 \times 10^{-8}$ aims to reduce the risk of chance positive results when conducting many statistical tests, but since the true effect sizes of most SNPs are small, the power to detect them is low unless thousands of samples are used.

Among GWAS of psychiatric disorders, schizophrenia is the flagship disorder having amassed large sample sizes faster than other disorders and so far identifying the most genome-wide significant associations. Early GWAS on schizophrenia had sample sizes of ~3,000 cases and found only one SNP passing the genome-wide significance threshold (International Schizophrenia Consortium et al., 2009). Increasing the sample size to over 17,000 schizophrenia cases identified 7 genetic associations (Schizophrenia Psychiatric Genome-Wide Association Study Consortium, 2011). In 2014, a landmark GWAS of over 36,000 cases and 113,000 controls, robustly linked 108 independent loci to schizophrenia, and this number has now increased to 143, with the inclusion of more samples (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014, Pardiñas et al., 2016). Increasing sample size by pooling data within international research consortia has been key to the success of GWAS and ten years of these

studies has led to about 10,000 strong genetic associations with complex traits (Visscher et al., 2017). The Psychiatric Genomics Consortium (PGC) was set up to conduct large-scale mega and meta-analyses of genome-wide genetic data and currently has working groups for nine psychiatric disorders (Sullivan et al., 2017).

1.3 Polygenic risk scoring

Genome-wide associations with disease can provide invaluable insights into biology, however these loci still only explain a small fraction of disease heritability (Wray et al., 2013). One reason for this is that common diseases have a polygenic architecture of many genetic variants with small effect sizes, which act cumulatively to increase risk. Simultaneously investigating thousands of SNPs, which individually do not reach statistical significance in a GWAS, could account for a greater amount of the heritability. Polygenic risk scoring is a method of testing the predictive power of such an ensemble of markers (International Schizophrenia Consortium et al., 2009). This uses association statistics from a discovery GWAS and after pruning SNPs for LD and weighting them according to their effect sizes, tests their combined predictive ability in an independent sample. Typically, LD pruning makes use of the P value informed clumping method, which preferentially retains the SNP with the strongest evidence of association within an LD window and removes other SNPs. For each SNP, the genotypes of individuals in an independent validation sample are weighted by the allele's effect size from the discovery GWAS and these effects are summed across multiple SNPs into a polygenic risk score (PRS). The PRS is tested for association with disease or control status in the validation sample using logistic regression with principal components as covariates. Often subsets of SNPs are selected according to a range of increasingly liberal P value thresholds, which can be lower than genome-wide significance. The proportion of variance in phenotype explained by the PRS usually starts to increase as the P value threshold is relaxed and more SNPs are added, consistent with the presence of small genetic effects that the original GWAS was underpowered to detect at the genome-wide significance level. At the P value threshold where the noise from additional null SNPs outweighs the predictive value of associated SNPs in the PRS, the amount of variance explained begins to decrease (International Schizophrenia Consortium et al., 2009, Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). A PRS derived from a discovery GWAS on one disease can also be tested for association with a different disease or trait in the validation sample, in order to investigate pleiotropy between them.

In the first application of polygenic risk scoring, PRS for schizophrenia explained ~3% of variance in schizophrenia status in an independent case-control sample, as well as ~2% of variance in a bipolar disorder case-control sample, demonstrating a shared polygenic component between these two disorders (International Schizophrenia Consortium et al., 2009). Notably, the power of a PRS is still dependent on the power of the discovery GWAS and as larger GWAS identify more true associations and estimate their effect sizes with greater accuracy, the amount of variance explained by polygenic risk scores will increase (Dudbridge, 2013). PRS generated from the latest schizophrenia GWAS results can now explain 11% of phenotypic variance in schizophrenia case-control samples (Pardiñas et al., 2016). Online GWAS summary association statistics for both psychiatric and physical disorders and the availability of software tools has enabled a range of informative research using polygenic risk scoring (Euesden et al., 2015, Purcell et al., 2007). The ultimate goal is to apply the method for genetic risk prediction in independent samples where the phenotype has not been measured, which could be used for preventative and precision medicine.

1.4 Genomic-relatedness-based restricted maximum-likelihood

Genomic-relatedness-based restricted maximum-likelihood (GREML) is an alternate polygenic method which estimates heritability based on all common genetic variants in a sample of unrelated cases and controls (Yang et al., 2011). A linear mixed model is used to compare each pair of individuals across all SNPs simultaneously, to estimate the proportion of the genome for which they have the same genotypes. This can be implemented in GCTA software and GREML was often referred to as GCTA in the early literature (Yang et al., 2011). SNP heritability (h^2_{SNP}) is the proportion of phenotypic variance attributable to the additive effects of genotyped and imputed SNPs and reflects a greater overall genetic similarity between cases than controls. A bivariate extension of the method can be used to calculate the SNP-correlation (r_g) between two traits, which can be measured in the same or different samples, to test for pleiotropy between them (Lee et al., 2012).

The power of GREML depends on the number of cases and controls in the sample. The h^2_{SNP} of schizophrenia calculated using GREML in a sample of ~9,000 cases and 12,000 controls was 23%, in contrast to the twin heritability estimate for schizophrenia which is 81% (Cross-Disorder Group of the Psychiatric Genomics Consortium et al., 2013, Sullivan et al., 2003). h^2_{SNP} estimates from well-powered datasets are usually a third to half of the twin heritability estimates, because h^2_{SNP} only captures the additive genetic effects of genotyped and imputed SNPs, whereas twin

heritability captures all genetic effects regardless of their frequency or complexity (Wray et al., 2013). Calculating h^2_{SNP} is useful in confirming the polygenic aetiology of a disease even when no genome-wide associations have been found and crucially, h^2_{SNP} represents the upper limit of genetic variance that could be explained by an infinitely powered GWAS.

1.5 Genetics of depression

Genome-wide association studies on MDD have lagged behind schizophrenia and other psychiatric disorders. In 2013, in a mega-analysis of over 9,000 clinically ascertained MDD cases and 9,000 healthy controls conducted by the PGC, no SNPs reached the genome-wide significance threshold (Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium et al., 2013). Studies of this sample size on most other human diseases had produced significant results. Several characteristics of MDD pose challenges to genetic analysis, for example its moderate heritability, high prevalence and the heterogeneity of depressive symptoms. Chapter 2 of this thesis is a review paper which discusses these characteristics in detail and outlines how they affect the statistical power of GWAS on MDD (Mullins and Lewis, 2017). Careful consideration of these features has informed more recent study designs and the last three years has seen the first genome-wide significant loci for depression identified through several novel approaches. Briefly, the CONVERGE consortium adopted the strategy of reducing heterogeneity and identified two genetic associations by focusing on a sample of Chinese women with recurrent severe depression (CONVERGE consortium, 2015). Three other loci have been found in Europeans by combining cohorts with clinical diagnoses of MDD and measures of depressive symptoms to increase sample size (Okbay et al., 2016, Direk et al., 2016). The direct-to-consumer genetic testing company 23andMe, identified 15 loci associated with depression using self-report of clinical diagnosis in a study of over 300,000 individuals (Hyde et al., 2016). Chapter 2 describes these GWAS designs and summarises the current state of the field in depression genetics (Mullins and Lewis, 2017).

1.6 Gene-by-environment interactions

Along with the main effects of genetics and environment, gene-by-environment interactions (GxE) whereby genetic effects are moderated by specific environmental factors, may also play a role in depression (Halldorsdottir and Binder, 2017). Many individuals who are exposed to environmental stressors do not become depressed and it has been suggested that this may

depend on their underlying genetic susceptibility. To date, GxE research in psychiatry has generally focused on candidate genes and the field has been plagued by publication bias towards positive findings with subsequent lack of replication (Duncan and Keller, 2011). The most studied GxE in depression is between the serotonin transporter promoter polymorphism (*5-HTTLPR*) and stressful life events or childhood trauma (Caspi et al., 2003). Over a decade's worth of these studies has produced inconsistent results and recently a coordinated meta-analysis concluded a lack of evidence for these interactions (Culverhouse et al., 2017). Since the genetic liability for depression is now known to be polygenic, there has been a move towards testing for interactions between environmental factors and polygenic risk scores. In the first such analysis, a PRS for MDD was found to interact with childhood trauma to increase risk of depression in adulthood, using data from the Netherlands Study of Depression and Anxiety (NESDA) (Peyrot et al., 2014). Musliner et al. (2015) found no evidence of interaction between PRS for MDD and adult stressful life events in the Health and Retirement Study. In the same sample, the first protective polygenic interaction was reported, whereby PRS for well-being buffered against the effect of bereavement on depressive symptoms (Domingue et al., 2017).

One limitation of these analyses is that interactions with PRS test for genome-wide moderation of genetic effects by the environment, while it is possible that interactions only occur at SNPs with particular functions. Given the lack of success of candidate gene interaction studies, genome-wide gene-by-environment interaction studies (GWEIS) are now being conducted, which systematically test interactions at every SNP (Dunn et al., 2016, Otowa et al., 2016). These polygenic approaches are a considerable advance and interactions warrant further investigation in depression samples with high-quality environmental data, which can now be more expensive and difficult to attain than genotypes. GWAS usually ignore the environment, including cases and controls with heterogeneous exposures and thus analysing SNP effects across average environmental backgrounds. Reducing environmental heterogeneity could be a useful strategy to increase effect sizes and uncover more genetic associations, which are missed by conventional GWAS. Understanding the interplay between genes and environment can further elucidate the pathophysiology of depression and could potentially target treatment and intervention strategies towards subgroups of patients with different combinations of risk factors.

1.7 Reproductive fitness

A key feature of depression and other psychiatric disorders is that affected individuals have significantly fewer children compared with the general population (Power et al., 2013a). This along with the substantial heritability and prevalence of psychiatric disorders is an evolutionary paradox, since natural selection should deplete genetic variation associated with reduced reproductive fitness (Keller and Miller, 2006). The mechanisms by which these genetic variants are maintained is unknown but several evolutionary theories have been proposed to explain this. Mutation-selection balance postulates that selection against deleterious sequence variants is balanced by the continuous occurrence of new mutations (Uher, 2009, Keller and Miller, 2006, van Dongen and Boomsma, 2013). Balancing selection suggests that variants that predispose to psychiatric disorders may confer reproductive advantage under some circumstances, compensating for negative selection against their otherwise deleterious effects on phenotypes (Uher, 2009, Keller and Miller, 2006, van Dongen and Boomsma, 2013). This would provide a mechanism through which GxE may play a role in enabling variants to evade negative selection. A third possibility is an accumulation of common variants with effects on psychiatric disorders that are individually too weak to be effectively targeted by negative selection in most human populations (Schork et al., 2009). A recent paper reported that common schizophrenia risk alleles can persist through background selection, a process by which negative selection against strongly deleterious variants, reduces the efficiency of selection against mildly deleterious variants at linked sites (Pardiñas et al., 2016). Most studies on the evolutionary paradox have examined the fitness of affected patients or their family members, but it is now possible to start empirically testing these evolutionary theories using genetic data in large-scale case-control or population samples. Natural selection shapes the genetic landscape of heritable traits and understanding the selection pressures on the genetic variants for psychiatric disorders can provide insight into their number, frequencies and effect sizes and inform future gene-mapping efforts.

1.8 Suicidality

In addition to reduced reproductive fitness, psychiatric disorders are associated with elevated all-cause and suicide mortality (Chesney et al., 2014). Suicidality is one of the core symptoms of MDD and ranges from suicidal thoughts, to plans, attempts and completed suicide. Suicide is a worldwide public health problem with over 800,000 deaths each year and suicide attempts occurring up to 20 times more frequently (World Health Organization, 2014). These stark figures

highlight the urgent need for improved prevention and treatment for suicidality. Currently, there are no robust methods of assessing suicide risk and clinicians must often rely on self-report from patients who are reluctant to disclose this information.

Over 90% of suicide attempters or victims have a psychiatric diagnosis, particularly depression and bipolar disorder (Qin, 2011, Beautrais et al., 1996). Suicide attempt aggregates in families and twin and family studies consistently indicate that the genetic aetiology of suicide attempt is partially distinct from that of the psychiatric disorders themselves (Brent and Mann, 2005). This could explain why some patients make suicide attempts, while the majority of psychiatric patients do not. Twin heritability estimates of suicidal behaviour are 30-55%, although one study showed that after adjusting for psychiatric disorders this reduced to 17% (Voracek and Loibl, 2007, Fu et al., 2002). Consistent with a diathesis-stress model, suicide attempt may be considered conditional on, but independent of psychiatric disorders. This epidemiological evidence suggests that suicidality could be usefully considered as a comorbidity rather than an intrinsic component of the psychiatric disorders themselves. Indeed, the DSM-5 lists Suicidal Behavior Disorder as a condition for further study and this nomenclature and clear criteria should lead to improved identification and documentation of this serious condition (American Psychiatric Association, 2013).

Several GWAS on suicide attempt have been conducted, by comparing attempters versus non-attempters with mood disorders, to test for genetic variants contributing independently to suicide attempt (Willour et al., 2012, Schosser et al., 2011, Perlis et al., 2010, Mullins et al., 2014b). These studies have failed to identify any replicable genetic associations, and with sample sizes of less than 1,200 cases, this is likely explained by a lack of power to detect the small genetic effects typical for a single SNP. Other GWAS have focused on subjects recruited specifically on the basis of suicide attempt, rather than a particular psychiatric disorder, but again have not found any genetic associations (Galfalvy et al., 2015). However, a PRS for suicide attempt generated from the results of one GWAS, showed modest association with suicide attempt in an independent sample, providing evidence that suicide attempt is a polygenic trait (Mullins et al., 2014b). Even genetic associations with small effects could provide enormous insights into the biological aetiology of suicidality and could lead to the development of much-needed treatments.

1.9 Summary and outline of thesis

This thesis aims to dissect the genetic and environmental heterogeneity of MDD using a range of statistical genetics methods in the largest available clinical and population samples. Chapter 2 provides a detailed review of the challenges that have faced depression genetics and the successful strategies adopted by recent GWAS (Mullins and Lewis, 2017). The first progress has been made in identifying genetic variants involved in depression with studies amassing the critical sample size necessary to reach an inflection point, beyond which the number of genetic associations is expected to increase linearly. The overwhelming evidence is that depression is a heterogeneous disorder in terms of symptoms, genetics and environmental risk factors, but leveraging this heterogeneity can make rapid inroads into understanding its complex aetiology. In the first research paper (Chapter 3), I investigate interactions between PRS for MDD and childhood trauma or adult stressful life events, using data from the Radiant UK sample of recurrent depression cases and healthy controls (Mullins et al., 2016).

Unaffected individuals will also carry alleles that increase risk of psychiatric disorders and studying genetic variation at a population level can provide insight into genetic architecture. In collaboration with deCODE genetics, Chapter 4 examines whether polygenic risk scores for psychiatric disorders are associated with number of offspring in unaffected individuals in the Icelandic population (Mullins et al., 2017). This study serves to increase our understanding of the selection pressures on these common genetic variants.

In the last two chapters of the thesis, I investigate the genetics of suicidality, a serious and understudied comorbidity of depression and other psychiatric disorders. Chapter 5 presents the largest GWAS on suicide attempt in over 6,000 attempters and 17,000 non-attempters with MDD, bipolar disorder and schizophrenia, recruited from the PGC. This is the first consortium-based GWAS on suicide attempt and makes progress in recruiting the large sample sizes needed for robust genetic studies on this phenotype. There is a pressing need for biomarkers, preventions and treatments for suicidality but small studies produce findings which do not replicate or have clinical utility. In Chapter 6, I follow up on a high-profile publication reporting that changes in the blood expression levels of four genes, *SAT1, PTEN, MAP3K3* and *MARCKS,* are biomarkers of high versus low suicidality state (Le-Niculescu et al., 2013). I conduct a direct replication of this hypothesis in the Genome-Based Therapeutic Drugs for Depression (GENDEP) study, a 12-week clinical trial of antidepressant treatment, with rich longitudinal phenotype data and gene expression measured at baseline and during treatment (Mullins et al., 2014a).

Increases in sample size have led to vast progress in depression genetics, however many questions about the aetiological heterogeneity of MDD remain unresolved. This thesis attempts to fill some of the gaps in our knowledge by exploring the complex interplay between genes and environment, the selection pressures on genetic variants for psychiatric disorders and the molecular genetic basis of suicidality.

2. Genetics of depression: progress at last

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GENETIC DISORDERS (F GOES, SECTION EDITOR)

Genetics of Depression: Progress at Last

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Abstract
Purpose of Review We will describe the success of recent genome-wide association studies that identify genetic variants associated with depression and outline the strategies used to reduce heterogeneity and increase sample size.
Recent Findings The CONVERGE consortium identified two genetic associations by focusing on a sample of Chinese women with recurrent severe depression. Three other loci have been found in Europeans by combining cohorts with clinical diagnosis and measures of depressive symptoms to increase sample size. 23andMe identified 15 loci associated with depression using self-report of clinical diagnosis in a study of over 300,000 individuals.
Summary The first genetic associations with depression have been identified, and this number is now expected to increase linearly with sample size, as seen in other polygenic disorders. These loci provide invaluable insights into the biology of depression and exciting opportunities to develop new biomarkers and therapeutic targets.

This article is part of the Topical Collection on *Genetic Disorders*

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Introduction

Major depressive disorder (MDD) is a common psychiatric illness and global public health problem [1]. It is the third leading cause of years lived with disability worldwide and a major contributor to early mortality from suicide [2]. Alleviating the burden of this costly disease is an important priority; however, limited understanding of the biological basis of depression has hindered the development of novel treatments and interventions.

Depression is a complex disorder with a heritability of 37% estimated from twin studies [3]. Despite robust evidence for a genetic component, identifying the specific genetic variants involved in the disorder has been a major challenge. Genome-wide association studies (GWAS) test differences in allele frequencies between disease and control groups at millions of common single nucleotide polymorphisms (SNPs) across the genome. These differences may be functionally relevant to the disease or may represent loci which are transmitted in linkage disequilibrium with a causative polymorphism. Early GWAS studies of MDD were not promising, despite having sample sizes similar to successful studies for other common diseases and traits, including psychiatric disorders. In a GWAS of over 9000 clinically ascertained MDD cases and 9000 healthy controls conducted by the Psychiatric Genomics Consortium (PGC), no SNPs reached the genome-wide significance threshold [4]. The CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) Consortium conducted a GWAS of depressive symptoms in

over 30,000 individuals which also failed to identify any genetic associations [5].

Challenges of Depression Genetics

There are several reasons why identifying risk loci for MDD has proven difficult. First, like most complex diseases, depression is a polygenic disorder arising from the combined effect of many genetic variants with individually small effect sizes [6]. Several sources provide evidence for the polygenic architecture of depression, despite a lack of genome-wide significant loci. Polygenic risk scoring uses association statistics from a discovery GWAS to weight the genotypes of individuals in an independent test sample and sums these effects across multiple SNPs into a polygenic risk score (PRS) [7]. Differences in PRS between cases and controls in the independent sample show that the PRS is capturing genetic susceptibility that is predictive of disease status. PRS for MDD generated from results of the PGC GWAS showed modest, although significant prediction for depression in independent samples ($R^2 = 0.6\%$, $P < 10^{-6}$), consistent with the presence of small genetic effects that the original GWAS was underpowered to detect at genome-wide significance [4]. SNP heritability (h^2_{SNP}) is the proportion of trait variance attributable to common SNPs and reflects a greater overall genetic similarity between cases than controls [8]. The h^2_{SNP} of MDD in the PGC GWAS was 0.21 (s.e. 0.021) [4, 9], again confirming the polygenic etiology of MDD. Large sample sizes are essential to detect small individual genetic effects, and pooling samples within research consortia has been key to the success of GWAS on many human traits.

The second characteristic of MDD which poses challenges to genetic analysis is its high lifetime prevalence of ~15% [1]. For a common disorder, the mean difference in phenotypic liability between case and control groups is smaller, for both unscreened and screened controls, and thus power to detect allele frequency differences between them is reduced. Power calculations show that samples 2.4-fold larger are needed for GWAS of MDD compared with schizophrenia (prevalence 1%), to identify a variant that explains the same proportion of risk [10]. Third, the heritability of MDD is modest, at 37%, compared with other psychiatric disorders, meaning that risk alleles are likely to have smaller effect sizes [3, 11]. To account for this lower heritability, samples 4–5 times larger would be required for MDD than schizophrenia to capture an equal amount of genetic variance [10].

Finally, depression is a particularly heterogeneous disorder. Some genetic heterogeneity is inherent to polygenicity; affected individuals may have different combinations of risk alleles and unaffected individuals will also carry many of these variants. But subphenotyping of the nine core symptoms of MDD indicates that almost 1500 symptom combinations can fulfill the diagnostic criteria and that two patients with a diagnosis of MDD may not have a single symptom in common [12]. Subtypes of depression such as recurrence or early-onset may be more heritable [3, 13]. Another striking example of heterogeneity is sex differences, with depression twice as prevalent among women than men and twin studies indicating that ~45% of the genetic liability to MDD is not shared between sexes [14–16]. Polygenic risk scoring methods also enable us to look for genetic similarities across traits and suggest that postpartum depression may be more genetically similar to bipolar disorder, that typical depression shows more pleiotropy with schizophrenia, and that atypical depression, characterized by increased appetite and weight, additionally shares genetic effects with BMI [17, 18]. These findings together provide compelling evidence that depression is likely composed of subtypes with differences in biological etiology and a heterogeneous genetic architecture. Therefore, the successful identification of genetic associations with MDD requires either increased sample sizes or empirically driven efforts to reduce heterogeneity. This review will outline recent genetic studies on depression which have adopted such strategies. Studies are described in detail, showing how each has advanced our understanding of the genetic underpinnings of depression, with summary information presented in Table 1.

CONVERGE Consortium

The CONVERGE (**C**hina, **O**xford and **V**irginia Commonwealth University **E**xperimental **R**esearch on **G**enetic **E**pidemiology) Consortium has collected a large depression cohort with detailed clinical, genetic and environmental data that is a powerful resource to dissect the etiology of depression [19, 20•]. The study aimed to ascertain a more homogeneous sample by restricting the phenotype to recurrent severe depression in women. Using low-coverage sequencing of 5303 Han Chinese MDD cases and 5337 controls screened to exclude MDD, two SNPs on chromosome 10 showed evidence of association: one near the *SIRT1* gene and the other in an intron of *LHPP* [20•]. Both loci replicated in an independent Chinese sample and the genetic signal at the *SIRT1* locus increased when further restricting the sample to melancholia, a more severe subtype of MDD [20•]. This study demonstrates the value of focusing on a homogeneous phenotype where genetic effects should be larger and easier to detect, even at the expense of a smaller sample size. SIRT1 is involved in the biogenesis of mitochondria, which are the cell's energy-producing organelles. Supporting the genetic association, the CONVERGE consortium report increased mitochondrial DNA in MDD cases versus controls, with the amount of increase positively correlated with stressors such as childhood sexual abuse and lifetime adverse events [21].

Table 1 Recent genome-wide association studies on depression

Study	Year	Total N	Cases	Controls	Cohort	Ancestry	Depression phenotype	GWAS hits	Putative genes	h^2_{SNP} (s.e.)
PGC [4]	2013	18,759	9240	9519		European	Lifetime MDD established using structured clinical interviews	0	–	0.21 (0.021)[a]
CHARGE [5]	2013	34,549			34,549	European	Depressive symptoms in past weeks assessed by questionnaires	0	–	Not calculated
CONVERGE [20•]	2015	10,640	5303	5337		East Asian	Recurrent MDD in women	2	*SIRT1, LHPP*	0.21 (0.030)
SSGAC [24•]	2016	180,866	16,471	58,835	105,739	European	Depressive symptoms in past 2 weeks assessed by 2 questions; lifetime MDD	2	*KSR2, DCC*	0.04 (0.004)
23andMe [22•]	2016	307,354	121,380	338,101		European	Self-report of diagnosis or treatment for major depression	17	*TMEM161B-MEF2C, VRK2, L3MBTL2, NEGR1, RERE, HACE1-LIN28B, SORCS3, OLFM4, PAX5, MEIS2-TMCO5A, intergenic, RSRC1-MLF1, intergenic, SLC6A15, NEGR1, KIAA0020-RFX3*	0.06[b]
CHARGE + PGC [32•]	2016	70,017	9240	9519	51,258	European	Depressive symptoms in past weeks assessed by questionnaires; lifetime MDD	1	*FHIT*	0.30 (0.040)

MDD major depressive disorder

[a] On the liability scale, given a prevalence of 15%

[b] In the discovery cohort, on the liability scale, given a prevalence of 25%

Although these genetic associations are a considerable step forward, the variants identified in individuals of East Asian ancestry have low frequencies in populations of European ancestry, and therefore no replication in the PGC depression samples or other studies has been achieved [20•, 22•]. The trans-ancestry genetic correlation between the PGC and CONVERGE GWAS results is ~0.3, indicating there are likely population differences in the genetic etiology of MDD, a finding with important implications for future studies [23]. Further comparison of the studies using genetic correlation and polygenic risk scoring weakly supports an overlap of SNP effects between the studies and strengthens when focusing on female only and recurrent MDD cases from the PGC [23]. This indicates that some of the genetic differences between the PGC and CONVERGE results may be due to differences in the specific MDD phenotype studied.

Social Science Genetic Association Consortium

The Social Science Genetic Association Consortium (SSGAC) has pursued the alternate strategy of increasing sample size, by analyzing multiple cohorts with heterogeneous measures of depression [24•]. They utilized data from two case-control studies of MDD: summary statistics from the PGC GWAS (9240 MDD cases, 9519 healthy controls) and dbGaP-accessible genotypes from the GERA (Resource for Genetic Epidemiology Research on Adult Health and Aging) study (7231 MDD cases, 49,316 controls) [4, 25]. These clinical samples were meta-analyzed with a GWAS on a measure of depressive symptoms in the UK Biobank, where adults in the general population were asked two questions about feelings of unenthusiasm or disinterest and depression or hopelessness in the past 2 weeks [26]. Combining these datasets resulted in a sample of 180,866 individuals and found two genome-wide significant associations with "depressive symptoms" which replicated on look up in an independent depression GWAS by 23andMe [24•]. One SNP is in the *KSR2* (kinase suppressor of ras 2) gene and the other is in the *DCC* gene, which encodes a transmembrane receptor involved in axon guidance. The h^2_{SNP} for depressive symptoms from the total sample was 0.04 (s.e. 0.004), which is considerably lower than the estimates from clinically ascertained MDD samples (~0.2 in both the PGC and CONVERGE studies) [9, 27]. This may result from mixing heterogeneous measures of depression which are influenced by different combinations of genetic variants and the weak information on depression symptoms from just two questions. Nevertheless, the SSGAC attributes the success of their study to exploiting the genetic correlation between clinical depression and depressive symptoms to combine studies and

increase sample size [24•]. While such a strategy may increase power for individual SNPs which influence both clinical depression and depressive symptoms, it may dilute associations for SNPs which only play a role in one phenotype and this has implications for replicating specific associations in different samples.

23andMe

The direct-to-consumer genetic testing company 23andMe (Mountain View, CA) also took the approach of increasing sample size. They used self-report data from consumers who participated in their research initiative and ascertained 75,607 individuals reporting previous clinical diagnosis or treatment for major depression and 231,747 individuals reporting no history of depression [22•]. They carried out meta-analysis of these results with the PGC GWAS results and then analyzed a replication sample of an additional 45,773 cases and 106,354 controls from 23andMe. A total of 17 independent SNPs from 15 regions reached genome-wide significance after joint analysis over all three data sets (Table 1) [22•]. Two of the loci were significant in both the meta-analysis and independent replication sample. In a locus spanning *MEF2C* (myocyte enhancer factor 2C) and *TMEM161B* (transmembrane protein 161B), two independent SNPs were significant. MEF2C is a transcription factor which plays a role in synaptic learning and memory and variants in the gene have been implicated in epilepsy, mental retardation, and schizophrenia [28–30]. The other locus encompasses the *NEGR1* gene, encoding neuronal growth regulator 1, which is involved in neurite outgrowth [31].

The strategy of less intensive phenotyping used in this study is a novel approach in psychiatric research, as cases have traditionally been ascertained using structured clinical interviews. To demonstrate the validity of the self-report measure, the authors calculated the genetic correlation between the results from the 23andMe study and those from the PGC GWAS. There was a high positive correlation of 0.72 (s.e. 0.09) between the results indicating common variant genetic overlap [22•]. However the h^2_{SNP} from the meta-analysis of the 23andMe discovery cohort and the PGC GWAS was 0.06, showing a substantially lower genetic component than the PGC h^2_{SNP} estimate of 0.21 [9, 22•]. This indicates that while the phenotypes are genetically correlated, the genetic signal in the 23andMe sample is likely weaker than in the PGC, which could reasonably be due to some diagnostic misclassification. The success of this 23andMe study in identifying genetic variants at genome-wide significance shows that large sample size can outweigh any reduction in power from additional heterogeneity or limited clinical information. Genotyping is now inexpensive compared with conducting detailed clinical interviews and 23andMe's light-phenotyping approach may be more likely to attract the large number of participants required in the absence of high-quality phenotype information.

CHARGE Consortium and PGC

Depression can be conceptualized along a spectrum of severity from subthreshold or minor depression to MDD of varying severity (e.g., mild, moderate, severe). Using a continuum approach to depression may augment statistical power because sample size can be increased substantially and individuals who fall anywhere along the phenotypic spectrum can be included. This was the rationale for combining the results of the CHARGE consortium GWAS of depressive symptoms and the PGC GWAS on MDD [32•]. Depressive symptoms were evaluated in individuals over 40 years old using validated questionnaires (mostly using the Center for Epidemiological Studies Depression Scale CES-D), which focused on depressive symptoms in the previous weeks rather than lifetime. This meta-analysis of a broad depression phenotype identified one genome-wide significant SNP, which replicated in an independent sample comprising newly ascertained MDD cases from the PGC and individuals assessed for depressive symptoms from the Health and Retirement Study [32•]. The SNP is located in an intron of *FHIT*, which is expressed in several brain regions and encodes a tumor suppressor protein also involved in oxidative stress and the circadian clock [32•].

In this study, the genetic correlation (r_g) between depressive symptoms and MDD was 1.00 (s.e. 0.2) which supports the concept of a depression continuum capturing similar genetic underpinnings to a study of depression cases and controls. Notably the h^2_{SNP} of the broad depression phenotype was 0.3 (s.e. 0.04), which was greater than the h^2_{SNP} of depressive symptoms or MDD separately (0.04 (s.e. 0.01) and 0.21 (s.e. 0.02), respectively) [32•]. Testing the genetic correlation between different phenotypic measures before combining them can be informative about heritability in the subsequent sample and can be used to assess whether the sample size achieved will be sufficient to outweigh any heterogeneity introduced.

Power and Study Design

The power of these studies to identify MDD-associated variants differs considerably by sample size and design. We calculated the genotype relative risk (GRR) which the study had 50% power to identify (Table 1), assuming a multiplicative model, allele frequency of 0.3, MDD prevalence of 15%, and fully screened controls [33]. The power of 50% was chosen to reflect the polygenic architecture of MDD, where many SNPs

of modest effect sizes contribute, and each study has low power to detect a specific variant, but higher power to detect a subset of SNPs having a pre-specified GRR. Using standard power calculations, the 23andMe study would have 50% power to detect a variant with GRR 1.024, but the PGC MDD study could only detect a GRR of 1.11. However, such power calculations make simplistic assumptions about study design, for example that selected participants are divided into MDD cases and controls (defined as non-cases), with cases generally being over-sampled from the population. In practice, studies such as CONVERGE and some PGC MDD cohorts select severe, recurrent cases of MDD and exclude any individuals with mild to moderate depression. This selection of severe cases and healthier controls with no history of depression increases the power of the study by inducing a larger difference in allele frequency between cases and controls. In contrast, study power will be reduced by any misclassification of cases and controls, which may be more likely in studies based on self-report or limited phenotypic information at a single time point.

Two of the studies listed in Table 1 use a quantitative phenotype of the number of depressive symptoms (SSGAC, CHARGE). The CHARGE study of 51,258 participants would have 50% power to detect a variant accounting for 0.0058% of trait variance. A study of 180,000 participants, similar to SSGAC, could detect a variant accounting for 0.017% of trait variance (with 50% power), but the SSGAC study used only two questions on depressive symptoms, reducing its power from this theoretical value.

The studies described here illustrate two approaches to dissect the genetic contribution to depression: through a case-control study of lifetime diagnosis of depression or using a continuous measure of the count of depressive symptoms, usually covering the previous 2 weeks. Although the time scales for these measures differ, the genetic correlation between these measures is high, for example r_g = 1 between CHARGE and the PGC MDD study [32•]. The relationship between the power of a case-control and continuous phenotype was derived by Yang et al. [34] and shows that a cohort study with a continuous phenotype on *N* individuals has lower power than a case-control study with *N*/2 cases and *N*/2 controls when the disease prevalence is below 10%. This validates the design of studies such as CONVERGE, ascertaining recurrent cases of MDD where the population prevalence in China is already low at 3.6% [35]. In Western countries where MDD prevalence is 15–20%, studies based on an underlying quantitative trait may have higher power than an equivalently sized case-control study.

Studies must balance the trade-off between gains in power from increased sample size or reduced heterogeneity. As the results of CONVERGE and the 23andMe studies show, both approaches can be successful in identifying genetic variants for depression, and researchers need to decide which strategy maximizes the use of their resources. Since depression is a common disorder, large sample sizes can be accrued through consortia and inventive new methods such as leveraging electronic medical records, population biobanks, and online recruitment. One limitation of mixing heterogeneous measures of depression or less intensive phenotyping is that any associations discovered may be more difficult to interpret. But the approach of increasing sample size can be used to find loci whose role in MDD can then be dissected in follow-up samples with more detailed phenotypic data, even if these have smaller sample size. Large samples with different depression phenotypes will help to disentangle the genetic background of different forms of depression.

Environment

While the focus of this review is on genetics, the role of the environment in depression cannot be ignored, with twin studies showing that it accounts for 63% of the variance [3]. In contrast to genetic associations, the environmental risk factors are well-established and include social isolation, unemployment, and relationship stressors [36]. Childhood abuse or neglect is one of the strongest environmental risk factors, more than doubling the risk for depression in adult life [37]. Gene-by-environment interactions (G×E) whereby genetic effects are moderated by specific environmental factors have long been postulated to play a role in depression. Most G×E research has focused on candidate genes such as the serotonin transporter promoter polymorphism (*5-HTTLPR*) interacting with stressful life events or childhood trauma. Over a decade's worth of studies on this interaction has produced inconsistent results, and recently, an extensive, pre-registered meta-analysis concluded a lack of evidence for the *5-HTTLPR* interaction with environmental adversity [38•].

Since the genetic liability for depression is known to be polygenic, studies have begun to test for interactions between environmental factors and polygenic risk scores, which capture the cumulative effect of many common variants in a single measure. To date, two studies have reported no interaction between PRS for MDD and adult stressful life events in the etiology of depression [39, 40]. Two studies have found significant interactions between PRS for MDD and childhood trauma, albeit in opposing directions [39, 41]. The reason for these discrepant results is unclear but further research is warranted as the detection of G×E has implications for future research strategies to identify genetic associations. In the Netherlands Study of Depression and Anxiety (NESDA), PRS had a stronger effect on MDD in individuals exposed to childhood trauma, which suggests that focusing on exposed individuals could render genetic effects larger, more homogeneous and easier to detect [41]. However, in the RADIANT UK study, the effect of PRS on MDD risk was stronger in those

unexposed to childhood trauma, suggesting that more power could be leveraged from GWAS by focusing only on individuals not exposed to trauma, as these MDD cases may have a stronger genetic predisposition. In summary, the analysis of cohorts with heterogeneous environmental exposures may also contribute to the difficulty in identifying genetic associations with MDD. Thus far, SNPs have been analyzed across average environmental backgrounds in GWAS but reducing environmental heterogeneity could be a valuable strategy to increase genetic effect sizes. There is a need for depression samples with good quality environmental data, which now can be more expensive and difficult to attain than genotype data.

Conclusions

The first progress has been made towards identifying genetic variants involved in MDD with studies amassing the critical sample size necessary to reach an inflection point beyond which the number of genetic associations is expected to increase linearly with sample size [42•]. The critical goal of GWAS is to identify the biological pathways underpinning depression and even risk alleles with small effects could yield enormous insights. As sample sizes continue to increase, MDD GWAS will uncover more and more of the genetic architecture of this debilitating disorder, as we have seen in GWAS studies on schizophrenia [30]. The next challenge is to establish the molecular mechanisms by which GWAS loci mediate their effects and translate these into much-needed new biomarkers and therapeutic targets. We have turned the corner in identifying genetic variants for depression, and the next few years will bring exciting opportunities to turn biological findings into clinical tools.

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Compliance with Ethical Standards

Conflict of Interest Niamh Mullins and Cathryn Lewis each declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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3. Polygenic interactions with environmental adversity in the aetiology of major depressive disorder

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ORIGINAL ARTICLE

Polygenic interactions with environmental adversity in the aetiology of major depressive disorder

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Background. Major depressive disorder (MDD) is a common and disabling condition with well-established heritability and environmental risk factors. Gene–environment interaction studies in MDD have typically investigated candidate genes, though the disorder is known to be highly polygenic. This study aims to test for interaction between polygenic risk and stressful life events (SLEs) or childhood trauma (CT) in the aetiology of MDD.

Method. The RADIANT UK sample consists of 1605 MDD cases and 1064 controls with SLE data, and a subset of 240 cases and 272 controls with CT data. Polygenic risk scores (PRS) were constructed using results from a mega-analysis on MDD by the Psychiatric Genomics Consortium. PRS and environmental factors were tested for association with case/control status and for interaction between them.

Results. PRS significantly predicted depression, explaining 1.1% of variance in phenotype ($p = 1.9 \times 10^{-6}$). SLEs and CT were also associated with MDD status ($p = 2.19 \times 10^{-4}$ and $p = 5.12 \times 10^{-20}$, respectively). No interactions were found between PRS and SLEs. Significant PRSxCT interactions were found ($p = 0.002$), but showed an inverse association with MDD status, as cases who experienced more severe CT tended to have a lower PRS than other cases or controls. This relationship between PRS and CT was not observed in independent replication samples.

Conclusions. CT is a strong risk factor for MDD but may have greater effect in individuals with lower genetic liability for the disorder. Including environmental risk along with genetics is important in studying the aetiology of MDD and PRS provide a useful approach to investigating gene–environment interactions in complex traits.

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Key words: Depression, genetics, gene–environment interactions, polygenic risk scoring.

Introduction

Major depressive disorder (MDD) is a global public health problem and the second leading cause of disability worldwide (Vos *et al.* 2012). The disorder has a well-established genetic contribution, with a heritability of 37% (Sullivan *et al.* 2000). Genome-wide association studies (GWAS) on depression have typically failed to identify the specific genetic variants involved (Ripke *et al.* 2013), although two loci have recently been implicated in the CONVERGE study of Chinese women with severe MDD (CONVERGE Consortium, 2015). Many environmental risk factors also increase the risk of depression, including unemployment, social

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isolation and relationship stressors (Brown & Harris, 1978). Stressful life events (SLEs) can trigger new depressive episodes and childhood trauma (CT) has been shown to double the risk for depression in adulthood (Kessler, 1997; Kendler *et al.* 1999; Nanni *et al.* 2012).

Gene–environment interactions (GxEs) whereby a person inherits sensitivity to environmental factors could also play an important role in MDD (Uher, 2014). GxEs have commonly been investigated using single loci in candidate genes, for example the serotonin transporter promoter polymorphism (*5-HTTLPR*) and its interaction with SLEs in major depression (Caspi *et al.* 2003). Despite this being the most widely investigated GxE in psychiatry, numerous studies including meta-analyses have produced discrepant results (Risch *et al.* 2009; Karg *et al.* 2011; Uher, 2014). There is more consistent evidence for an interaction between *5-HTTLPR* and CT, conferring risk for persistent depression in adulthood (Karg *et al.* 2011; Uher *et al.* 2011; Brown *et al.* 2013; Fisher *et al.* 2013). Nevertheless, the conflicting results from GxE studies in psychiatry have made these findings controversial (Duncan & Keller, 2011).

Further analyses of the genetic effects on MDD have indicated that the disorder is likely to be highly polygenic, arising from the combined effect of many risk variants, each with small effect sizes (Wray *et al.* 2012; Ripke *et al.* 2013). Polygenic risk scoring can be used to test the predictive power of multiple genetic variants simultaneously. Subsets of single nucleotide polymorphisms (SNPs) from a discovery GWAS are selected according to their *p* value and weighted by their effect size to create a polygenic risk score (PRS) for each individual in an independent validation sample. The PRS can then be tested for its ability to differentiate between case and control status in the validation dataset (Purcell *et al.* 2009; Dudbridge, 2013). PRS derived using results from the largest GWAS on MDD by the Psychiatric Genomics Consortium have shown significant predictive ability for depression, explaining about 0.9% of variance in case–control samples (Ripke *et al.* 2013; Peyrot *et al.* 2014).

These findings have led to the hypothesis that GxEs in a highly polygenic trait such as MDD may involve multiple genetic variants rather than one specific locus. Indeed, interactions between polygenic scores for MDD and CT were found to increase risk for depression in the Netherlands Study of Depression and Anxiety (NESDA), accounting for 0.6% of variance in MDD status (Peyrot *et al.* 2014). The dearth of significant results in GWAS of MDD may be partially due to environmental influences not being accounted for and investigation of GxEs could provide important insights into the complex aetiology of the disorder. Here we test for interactions between polygenic risk for major depression and adult SLEs or CT in the RADIANT UK study of recurrent MDD.

Method

Clinical sample collection

Depression cases ($n = 1605$) were drawn from three studies previously described in the published literature. The RADIANT UK recurrent MDD sample is comprised of the Depression Case Control (DeCC) study and probands from the Depression Network (DeNT) study of affected sibling pairs (Farmer *et al.* 2004; Cohen-Woods *et al.* 2009). UK-ascertained cases from the Genome Based Therapeutic Drugs for Depression (GENDEP) study were also included. GENDEP is a prospective pharmacogenetic study of patients with unipolar depression of at least moderate severity, on a 12-week antidepressant treatment (Uher *et al.* 2010). Briefly, patients were diagnosed using the Schedules for Clinical Assessment in Neuropsychiatry Interview, according to standardized criteria (Wing *et al.* 1990). Information was recorded on patients' worst and second worst episodes of depression in the DeCC and DeNT studies and on their current episode in the GENDEP study (Lewis *et al.* 2010). Exclusion criteria included personal or family history of other psychiatric diagnoses besides anxiety disorder (Farmer *et al.* 2004; Cohen-Woods *et al.* 2009; Uher *et al.* 2010).

Healthy controls ($n = 1064$) were available from the DeCC study and the London site of the Bipolar Affective Disorder Case–Control study (Gaysina *et al.* 2009; Lewis *et al.* 2010). Controls were screened for lifetime absence of all psychiatric disorders using the Past History Schedule (McGuffin *et al.* 1986). First-degree family history of any psychiatric disorder or a score of 10 or more on the Beck Depression Inventory at interview were further exclusion criteria (Beck *et al.* 1996; Cohen-Woods *et al.* 2009, 2010).

Replication analysis was conducted using recurrent depression cases from The Genetics of Recurrent Early-Onset Depression (GenRED) 1 study ($n = 260$), the GenRED 2 study ($n = 270$) and the Depression Genes and Networks (DGN) study ($n = 469$) (Shi *et al.* 2011; Battle *et al.* 2014). Individuals in all analyses were of white European parentage and gave written informed consent to participate. Further information on clinical samples is provided in the online Supplementary material.

Measures

Recurrent MDD was defined according to standard criteria, as having at least two episodes of moderate severity, separated by two or more months of remission (World Health Organization, 1993;

American Psychiatric Association, 1994). Number of episodes was not a requirement in the GENDEP study, although the majority of cases were recurrent (Lewis *et al.* 2010).

Whole-blood samples were collected in ethylene-diamine-tetra-acetic acid (EDTA) from depressed cases. DNA samples were collected from controls by taking blood or using buccal mucosa swabs returned via postal mail. DNA was extracted and samples of sufficient quantity and quality were genotyped on the Illumina Human610-Quad BeadChip (Illumina, Inc., USA) (Freeman *et al.* 2003; Lewis *et al.* 2010).

Adult SLEs were assessed using the Brief Life Event Questionnaire, which is a shortened version of the List of Threatening Experiences Questionnaire (LTE-Q) (Brugha *et al.* 1985). Childbirth was also included, giving a total of 12 items (Farmer *et al.* 2004) (online Supplementary material). Cases in the DeCC and DeNT studies were asked to report on whether or not they experienced each SLE in the 6 months prior to their worst episode of depression, while GENDEP cases were asked to report on the 6 months preceding the clinical trial (Keers *et al.* 2011; Fisher *et al.* 2012). Controls reported on the 6 months prior to their interview. The number of reported SLEs was summed for each individual and analysed as a quantitative variable with range 0–12. Following the LTE-Q categories, SLEs were split into those considered dependent on an individual's behaviour and those which seem independent (Brugha *et al.* 1985). Dependent SLEs included unemployment, separation, financial or legal difficulties and the birth of a baby. Independent events included personal illness, illness of a family member, death of a family member and being robbed. This gave a total of seven dependent and five independent SLEs (online Supplementary material). Mood at the time of interview was assessed using the self-report Beck Depression Inventory in the DeCC and GENDEP cases (Beck *et al.* 1996).

A subset of the sample ($n = 240$ cases, $n = 272$ controls) completed the self-report Childhood Trauma Questionnaire, which measures frequency and severity of sexual, physical and emotional abuse, physical and emotional neglect during childhood, using 25 Likert-type items (Bernstein *et al.* 2003). CT was firstly analysed as a quantitative score with range 25–125. To explore results, CT was divided into categories of none, mild and moderate/severe, according to a definition described previously in this sample (Fisher *et al.* 2013). The GenRED and DGN replication studies assessed CT with the self-report Childhood Events Questionnaire (E. Nelson and D. Levinson, unpublished observations), which is based on the US National Comorbidity Survey CT screening items (Kessler *et al.* 1997) and CT questionnaires from Washington University (Nelson *et al.* 2002). These items cover severity and frequency of sexual abuse, physical abuse and trauma (within and outside the family), and emotional neglect.

Quality control

Standard quality-control procedures were implemented to clean genetic data, leaving 471 747 SNPs (Lewis *et al.* 2010). Principal components (PCs) were calculated using EIGENSTRAT (Price *et al.* 2006). The first two PCs reduced the genomic control parameter (γ) to 1.02, indicating little difference between RADIANT UK cases and controls due to population stratification or other systematic genomic effects (Lewis *et al.* 2010). Missing information on age at worst episode of depression (233 cases) and age at interview (34 controls) was replaced with the mean age at worst episode or interview in males or females as appropriate. Number of SLEs was significantly associated with age ($p = 3.64 \times 10^{-8}$) and sex ($p = 0.001$), with younger individuals and females reporting more SLEs. Since depressed cases were younger than controls and contained a greater proportion of females, the number of SLEs was adjusted in cases to remove bias due to age and sex. Using controls as a proxy for the general population, a linear regression of SLEs on age and sex was used to estimate their association. These regression coefficients were then used to adjust the number of SLEs in depressed cases. Dependent and independent SLEs were adjusted separately in the same manner. CT score was not associated with age or sex, so no adjustment was performed.

Statistical analysis

Polygenic scores were constructed using summary results available online from the Psychiatric Genomics Consortium (https://pgc.unc.edu/) MDD GWAS (Ripke *et al.* 2013). The RADIANT UK sample was removed to provide an independent validation dataset and a meta-analysis of the remaining eight studies was conducted (7615 cases and 7931 controls). These discovery GWAS results were pruned for linkage disequilibrium (LD) using the p value informed clumping method in PLINK v1.07 (http://pngu.mgh.harvard.edu/purcell/plink/), based on the LD structure from the RADIANT UK dataset (Purcell *et al.* 2007). Clumping preferentially retains SNPs with the strongest evidence of association and removes SNPs in high LD ($r^2 > 0.25$ within a 300 kb window, filtering for significance, PLINK-command: --clump-p1 0.5 --clump-p2 0.1 --clump-r2 0.25 --clump-kb 300). Subsets of SNPs were selected from the results at

nine increasingly liberal p value thresholds ($p_T < 0.0001$, $p_T < 0.001$, $p_T < 0.01$, $p_T < 0.05$, $p_T < 0.1$, $p_T < 0.2$, $p_T < 0.3$, $p_T < 0.4$, $p_T < 0.5$). These sets of alleles, weighted by their log odds ratios (ORs) from the discovery study, were summarized into PRS for each individual in the validation sample using PLINK (Purcell *et al.* 2009). $p_T < 0.5$ contained 87 737 SNPs.

PRS for MDD were tested for ability to predict case/control status in RADIANT UK using logistic regression in R (http://www.r-project.org) to calculate the Nagelkerke's pseudo-R^2 measure of variance explained, excluding the variance accounted for by two PCs (Nagelkerke, 1991). SLEs and CT score were also tested for association with case/control status. Interactions between PRS and SLEs or CT were investigated using two models. A multiplicative model tests interaction as departure from multiplicativity, meaning that the combined effect of PRS and environment differs from the product of their individual effects. This was tested using a logistic regression, co-varying for the main effects of PRS, environment and two PCs. Models were also adjusted for PC × environment and PC × PRS interactions (Keller, 2014). The interaction term was tested for its ability to differentiate between case and control status in the validation sample by calculating Nagelkerke's pseudo-R^2. An additive interaction model tests whether the combined effect of PRS and environment differs from the sum of their individual effects. It has been suggested that this better captures the biological mechanism of GxEs (Rothman *et al.* 2008). Interaction as departure from additivity was tested using linear regression of MDD case/control status on the interaction term, with covariates as described previously. The multiple-R^2 measure of variance explained by the interaction was calculated. To investigate gene–environment correlations, whereby genotypes may influence exposure to different environments, PRS for MDD were tested for association with SLEs or CT score using a linear regression, co-varying for two PCs. Empirical p values were calculated using permutation procedures for all analyses. Ten independent tests were conducted, giving a Bonferroni corrected significance threshold of 0.005. In the replication phase, gene–environment correlations between PRS and CT score in depressed subjects were tested in MDD cases from the independent GenRED and DGN samples.

Power calculations for our study were performed in QUANTO version 1.2.4 (Gauderman & Morrison, 2009), using ORs reported in the literature for the effects of PRS (OR = 1.22), 2+ SLEs (OR = 1.82) and CT (OR = 2.27) on MDD (Nanni *et al.* 2012; Motrico *et al.* 2013; Peyrot *et al.* 2014). The study had >80% power to detect an interaction between PRS and SLEs with an OR of 1.28 (at $\alpha = 0.05$). In the subset with CT data, there was >80% power to detect an interaction between PRS and CT with an OR of 1.76 (at $\alpha = 0.05$).

Table 1. *Sample characteristics*

	Cases ($n = 1605$)	Controls ($n = 1064$)	p[a]
Sex, *n* (%)			6.83×10^{-10}
Male	471 (29.3)	436 (41.0)	
Female	1134 (70.7)	628 (59.0)	
Age, years[b]	36.7 (12.3)	41.5 (13.2)	4.16×10^{-19}
Age of onset, years	23.1 (11.4)		
Number of episodes	2.48 (0.68)		
Number of SLEs[b]	1.57 (1.48)	0.68 (0.87)	1.67×10^{-67}
Number of dependent SLEs[b]	0.88 (1.05)	0.23 (0.51)	3.05×10^{-79}
Number of independent SLEs[b]	0.69 (0.89)	0.44 (0.66)	1.29×10^{-11}
Childhood trauma score[c]	46.31 (16.25)	32.75 (8.75)	7.13×10^{-26}

Data are given as mean (standard deviation) unless otherwise indicated.

SLEs, Stressful life events.

[a] p Values were calculated using a non-parametric Mann–Whitney U test, with the exception of sex where a χ^2 test was used.

[b] For cases at worst episode of depression and for controls at interview.

[c] Data were available on a subset of 240 cases and 272 controls. Statistics were calculated from individuals without missing data.

Ethical standards

All procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Results

Sample characteristics

Sample characteristics are shown in Table 1. Cases contained a significantly greater proportion of females than controls and their mean age at worst episode of depression was significantly younger than mean age at interview in controls. Cases had experienced significantly more SLEs and had higher CT scores than controls (Table 1). In the subset of the sample with CT data (240 cases and 272 controls), similar differences in sex and age at interview were found between cases and controls ($p = 2.9 \times 10^{-4}$ and $p = 0.004$, respectively).

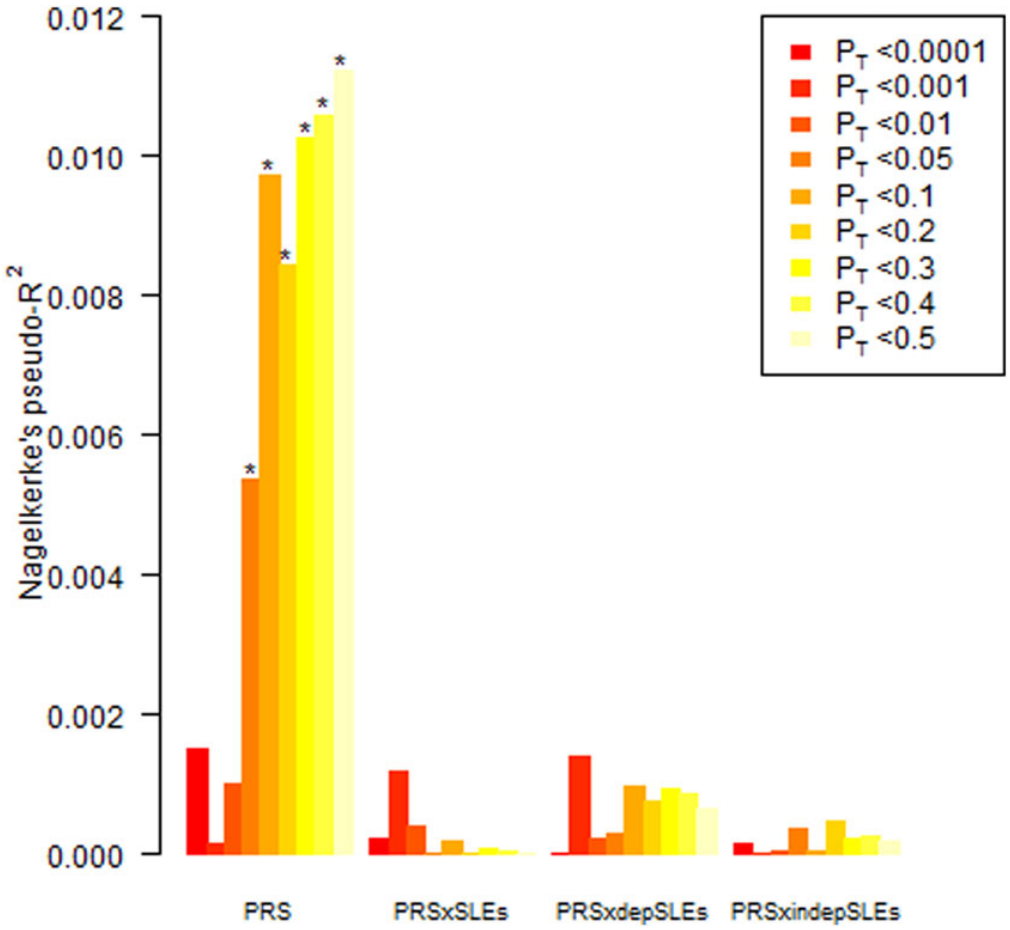

Fig. 1. Polygenic risk scores (PRS) for major depressive disorder and multiplicative interactions with stressful life events (SLEs) used to predict depression in the RADIANT UK sample. The y-axis indicates Nagelkerke's pseudo-R^2, a measure of the variance explained. On the x-axis the nine p value thresholds used to select single nucleotide polymorphisms in the discovery phase are plotted left to right. depSLEs, Dependent SLEs; indepSLEs, independent SLEs; p_T, p value threshold. * $p <$ 0.005. For a colour figure, see the online version.

Interaction with SLEs

Polygenic scores derived from a meta-analysis of MDD using data from the Psychiatric Genomics Consortium showed significant predictive ability for depression in the RADIANT UK sample. As more SNPs were added to the PRS at increasingly liberal p value thresholds, the amount of variance explained increased. At $p_T < 0.5$, the polygenic score explained 1.1% of variance in case/control status [$p = 1.9 \times 10^{-6}$, OR = 1.22, 95% confidence interval (CI) 1.12–1.32] (Fig. 1). After adjustment for age and sex, total SLEs were still significantly associated with MDD status ($p = 2.19 \times 10^{-4}$), explaining 0.7% of variance between cases and controls. A greater number of dependent SLEs was associated with case status and could predict 6.6% of variance in phenotype ($p = 1.35 \times 10^{-25}$). Independent SLEs showed significant but weaker predictive ability ($p = 1.36 \times 10^{-9}$, Nagelkerke's pseudo-$R^2 = 0.019$) and in contrast to dependent SLEs, more independent events were found in controls *v.* cases, after correction for age and sex. Under a multiplicative model, there was no interaction between the PRS and total number of SLEs (Fig. 1). The largest R^2 was 0.001 at $p_T < 0.001$ ($p = 0.12$, OR = 1.05, 95% CI 0.98–1.12). Interactions between PRS and dependent or independent SLEs were also non-significant (Fig. 1). No interactions were found under additive models (online Supplementary material).

Interaction with CT

In the subset of the sample with CT data, the PRS did not show significant predictive ability for MDD ($p =$ 0.078, Nagelkerke's pseudo-$R^2 = 0.007$, $p_T < 0.4$), though effects were in the expected direction (OR = 1.18, 95%

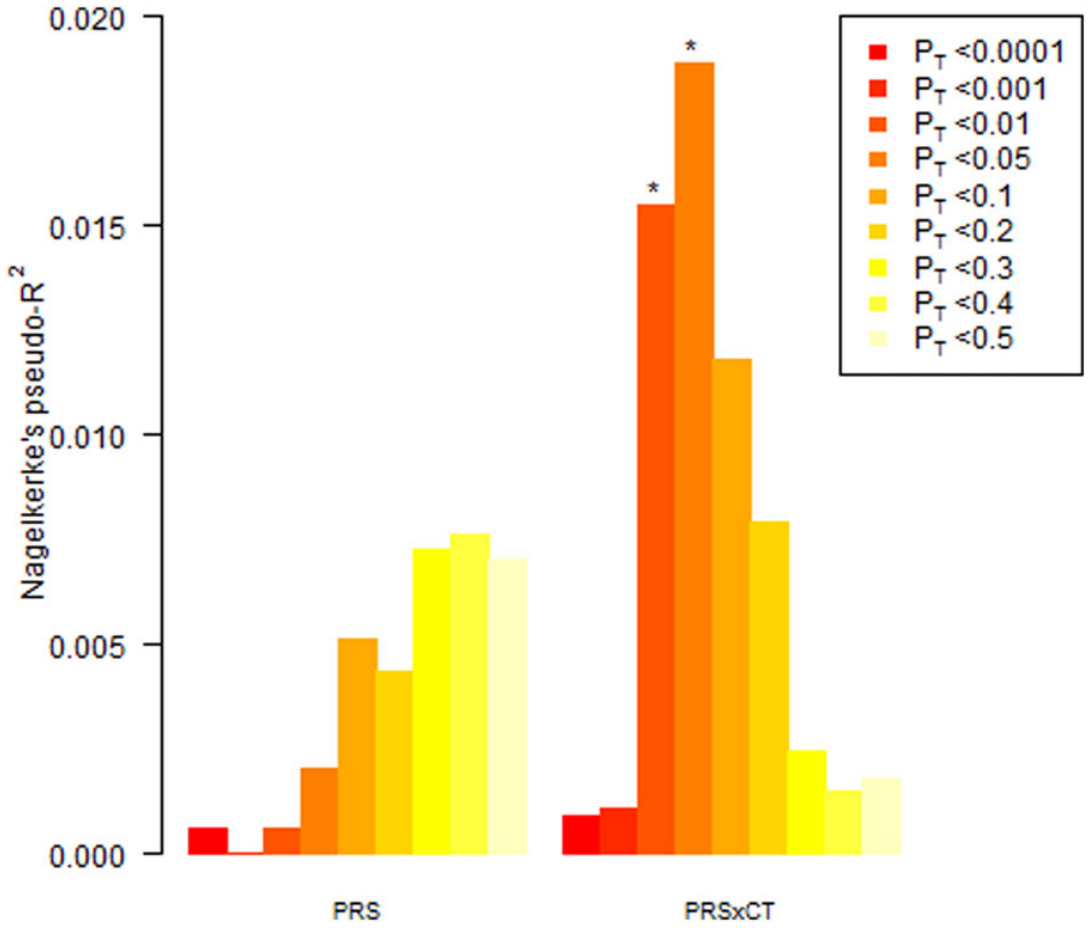

Fig. 2. Polygenic risk scores (PRS) for major depressive disorder and multiplicative interaction with childhood trauma (CT) used to predict depression in the RADIANT UK sample. The y-axis indicates Nagelkerke's pseudo-R^2, a measure of the variance explained. On the x-axis the nine p value thresholds used to select single nucleotide polymorphisms in the discovery phase are plotted left to right. p_T, p value threshold. * $p < 0.005$. For a colour figure, see the online version.

CI 0.98–1.42) (Fig. 2). A higher CT score was significantly associated with depression status, explaining 30.2% of variance ($p = 5.12 \times 10^{-20}$). Multiplicative interactions were found between polygenic scores for MDD and CT (Fig. 2). The interaction at $p_T < 0.05$ explained 1.9% of variance in the phenotype and was significant after multiple testing correction ($p = 0.002$). There was an inverse association between the interaction and MDD status (OR = 0.96, 95% CI 0.94–0.98). To visualize these results, interactions were plotted between categories of CT (none, mild, moderate/severe) and PRS standardized to mean 0 and S.D. 1. Plotting log odds of depression by polygenic score for each CT category at the p value threshold with most significant interaction ($p_T < 0.05$; $p = 0.002$) allows visualization of the results (Fig. 3). For individuals who had not experienced CT, a higher PRS for MDD was associated with a higher risk of the disorder (Fig. 3; black line). Individuals in the mild CT category were at an increased risk of depression but this appeared to act independently of their genetic liability (mid-grey line). Those who had experienced moderate/severe CT were mostly depressed cases but interestingly the individuals at highest risk in this category had a lower PRS than average (Fig. 3; light grey line). There were no interactions between PRS and CT under additive models (online Supplementary material).

Gene–environment correlations

Gene–environment correlations were explored between PRS for MDD and number of SLEs. Significant correlations were found within the MDD cases, specifically with dependent ($p_T < 0.001$; $p = 0.002$) and not independent SLEs (online Supplementary material). No significant corrections were found between polygenic score and CT score in the RADIANT UK sample (online Supplementary material). As the interaction

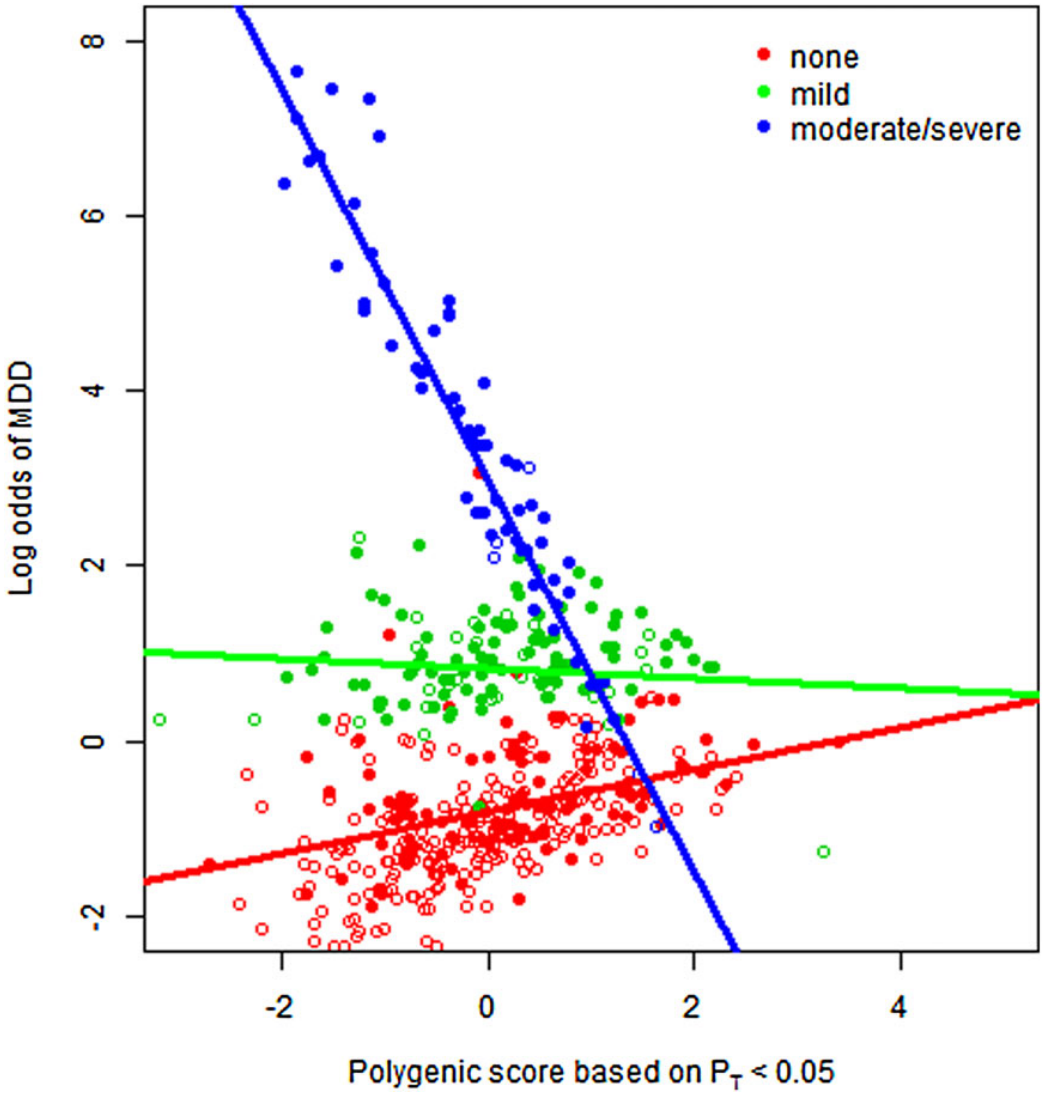

Fig. 3. Multiplicative interaction between standardized polygenic risk score for major depressive disorder (MDD) based on $p_T < 0.05$ and categories of childhood trauma. Shaded circles are cases and open circles are controls. p_T, p value threshold. For a colour figure, see the online version.

between PRS and CT showed an inverse association with MDD status, the relationship between PRS and CT score was tested in the independent GenRED and DGN depression cases. No significant correlations between PRS and CT score were found.

Mood at interview

To investigate whether low mood at the time of interview may result in a recall bias for negative events, cases severely depressed at interview were removed in a sensitivity analysis. Gene–environment correlations between PRS and dependent SLEs in cases were no longer significant, excluding those who were severely depressed at interview (online Supplementary material). Interactions between PRS and CT score remained significant after severely depressed cases were excluded (online Supplementary material).

Discussion

Polygenic scores derived from the Psychiatric Genomics Consortium MDD mega-analysis predicted depression in the RADIANT UK sample, explaining 1.1% of variance in case/control status. This modest figure is in line with previous estimates from this mega-analysis and confirms the presence of associated variants that

the original GWAS was underpowered to detect (Ripke *et al.* 2013; Peyrot *et al.* 2014). SLEs were also significant predictors of case/control status. We hypothesized that given the polygenicity of MDD, testing interactions between polygenic scores and environmental adversity could be a more powerful approach than using single genetic variants in a candidate gene. No interactions were found between PRS for MDD and total, dependent or independent SLEs, which is in agreement with previous findings by Musliner *et al.* (2015).

In the subset of the sample with CT data, the PRS failed to show significant predictive ability for depression which probably reflects the restricted sample size. Consistent with previous reports, CT was a strong risk factor for recurrent MDD in adulthood (Nanni *et al.* 2012). Significant interactions were found between PRS and CT; however, there was an inverse association with depression status. This appeared to be driven by individuals who had experienced moderate/severe CT, as those at highest risk in this category tended to have a lower PRS than other cases or controls (Fig. 3). One possible explanation is that CT may be more important in the development of MDD for individuals who have a low genetic risk than for individuals who have a high genetic risk. This would be consistent with the liability threshold model for MDD, where a combined effect of many genetic risk variants together with an environmental contribution causes an individual to cross the liability threshold and become affected. Alternatively, the experience of CT may be such a strong risk factor for depression that genetics has a negligible effect.

In contrast to our results, the NESDA study found a significant PRS x CT interaction, whereby higher PRS and severe CT increased the risk for MDD (Peyrot *et al.* 2014). The conflicting results of these studies may be due to differences in design – for example, NESDA is a population-based study, includes single-episode, recurrent and chronic depression, used a different instrument for assessing CT and had a larger sample size (1645 MDD cases, 340 controls). It has been reported that interaction between *5-HTTLPR* and CT specifically increases risk for chronic depression in adulthood (Brown *et al.* 2013). This suggests that GxEs may be more specific than anticipated and subtle differences between our study and NESDA could have contributed to the discrepant results. Similarly, our SLE assessment was for the 6 months prior to the worst episode of depression in recurrent depression cases. Testing SLEs preceding the initial onset of depression or in single-episode depression may identify different components of the gene–environment aetiology of MDD.

Evidence of gene–environment correlation was found, as polygenic scores for MDD increased exposure to (or reporting of) dependent SLEs in MDD cases (online Supplementary material). This suggests that depressed individuals may select themselves into environmental adversity by creating stressful life events due to their own behaviour, which is known as active gene–environment correlation. SLEs or the reporting of SLEs is heritable (Power *et al.* 2013) and twin studies have shown pleiotropy between the genetic contribution to SLEs and genetic liability to depression (Kendler & Karkowski-Shuman, 1997; Silberg *et al.* 1999).

There are several strengths of this study. SLEs were adjusted for age and sex prior to the analyses. The amount of variance explained by the SLEs decreased dramatically after adjustment (online Supplementary material), which demonstrates the importance of accounting for age and sex. It has been suggested that low mood at the time of interview may cause recall bias for negative events; however, we found no evidence that this influenced our results, consistent with two previous analyses in the RADIANT UK sample (Fisher *et al.* 2012, 2013).

A number of limitations also warrant noting. The discovery GWAS by the Psychiatric Genomics Consortium was underpowered to detect the likely effect sizes in MDD, which reduces the ability to separate modest signals from noise and achieve accuracy in estimation of the PRS (Dudbridge, 2013; Ripke *et al.* 2013). The PRS used in these analyses consists of SNPs selected from a study of their main effect on MDD, which may not be the same genetic variants that are involved in GxEs. This could explain the non-standard shape of the PRS histograms for the interactions, in contrast to the usual pattern where variance explained increases across the p value thresholds (Figs 1 and 2). Our study design relied on retrospective self-reports of depression and environment, which may be less accurate if the events occurred a long time ago. However, the worst episode of depression is arguably the most memorable, and retrospective self-reports of depressive episodes agree well with hospital records (McGuffin *et al.* 1986; Kendler *et al.* 1993).

The detection of GxEs has implications for future research strategies. Analysis of cohorts with heterogeneous environmental exposures may partially explain the lack of success in detecting genetic associations with MDD. Our results suggest that more power could be leveraged from GWAS by focusing only on individuals not exposed to CT as this might identify 'more genetic' cases of MDD. However, results of the NESDA study suggest that focusing on exposed individuals could render genetic effects larger, more homogeneous and easier to detect (Flint & Kendler, 2014; Peyrot *et al.* 2014). Polygenic interactions in MDD require further investigation in larger, similarly well-characterized samples and could provide important insights into the complex aetiology of depression.

Supplementary material

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S0033291715002172

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Declaration of Interest

A.E.F. and P.M.G. have received consultancy fees and honoraria for participating in expert panels from pharmaceutical companies including Lundbeck and GlaxoSmithKline. All other authors declare that they have no conflicts of interest.

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Supplementary material

Polygenic interactions with environmental adversity in the aetiology of major depressive disorder

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Clinical Sample Characteristics

Participants in the DeCC and DeNT studies were identified in London, Cardiff and Birmingham from psychiatric clinics, hospitals, general medical practices and media advertisements (Cohen-Woods, 2009, Farmer, 2004). The GENDEP clinical trial was conducted across nine European centres and UK ascertained cases were included in these analyses (Uher, 2010). Patients were diagnosed using the Schedules for Clinical Assessment in Neuropsychiatry Interview (SCAN), according to standardised criteria in the International Classification of Diseases 10th edition (ICD-10) or Diagnostic and Statistical Manual 4th edition (DSM-IV) (American Psychiatric Association, 1994, Wing, 1990, World Health Organization, 1998). Table S1 shows the characteristics of depressed cases from the DeCC, DeNT and GENDEP studies separately. Healthy controls in the DeCC and BACCs studies were recruited through the Medical Research Council general practice research framework, newspaper advertisements or via internal emails at King's College London (Lewis, 2010, Gaysina, 2009).

Table S1: Characteristics of depressed cases from the DeCC, DeNT and GENDEP studies

	Cases with information on stressful life events			
Study	**DeCC (n=1232) (%)**	**DeNT (n=294) (%)**	**GENDEP (n=79) (%)**	**Total (n=1605) (%)**
Male (%)	375 (30.4)	69 (23.5)	27 (34.2)	471 (29.3)
Female (%)	857 (69.6)	225 (76.5)	52 (65.8)	1134 (70.7)
Mean age at interview (years) (s.d.)	46.7 (12.3)	45.6 (11.0)	44.7 (12.3)	46.4 (12.1)
Mean age at worst episode (years) (s.d.)	36.5 (12.2)	35.1 (11.6)	-	36.2 (12.1)
Mean age onset (years) (s.d.)	23.2 (11.5)	22.4 (10.4)	24.1 (14.6)	23.1 (11.4)
Mean number of episodes (s.d.)	2.8 (2.8)	3.1 (2.0)	3.4 (4.6)	2.48 (0.68)
Proportion with recurrent MDD	1232 (100)	294 (100)	62 (78.5)	1588 (98.9)
	Cases with information on childhood trauma			
Study	**DeCC (n=183) (%)**	**DeNT (n=57) (%)**	**GENDEP (n=0) (%)**	**Total (n=240) (%)**
Male (%)	50 (27.3)	13 (22.8)	-	63 (26.2)
Female (%)	133 (72.7)	44 (77.2)	-	177 (73.5)
Mean age at interview (years) (s.d.)	44.3 (13.0)	45.9 (10.7)	-	44.7 (12.5)
Mean age at worst episode (years) (s.d.)	34.2 (12.3)	34.1 (11.8)	-	34.2 (12.1)
Mean age onset (years) (s.d.)	21.6 (11.2)	19.7 (9.7)	-	21.1 (10.8)
Mean number of episodes (s.d.)	2.9 (2.5)	3.4 (2.2)	-	3.0 (2.8)
Proportion with recurrent MDD	183 (100)	57 (100)	-	240 (100)

DeCC - Depression Case Control study, DeNT - Depression Network study, GENDEP - Genome Based Therapeutic Drugs for Depression study, MDD - major depressive disorder

Depressed cases from the GenRED 1, GenRED 2 and DGN studies were used in replication analyses. Cases in the GenRED studies were recruited in clinical settings and through media and internet announcements and advertisements by six research groups at Stanford University, Columbia University, Johns Hopkins, Rush Presbyterian Medical Center Chicago, University of Iowa and University of Pittsburgh (Shi, 2011, Levinson, 2003). Cases were diagnosed using the

Diagnostic Interview for Genetics Studies 3.0, family informant if available was interviewed with the Family Interview for Genetic Studies and psychiatric records were obtained where possible (Nurnberger, 1994). Cases had recurrent (≥2 episodes) or chronic (≥3 years) MDD with onset before 31 years old. All subjects were of European ancestry. GenRED 1 cases were genotyped using the Affymetrix 6.0 genome-wide SNP array (Affymetrix, Santa Clara, USA) and GenRED 2 cases were genotyped using the Illumina HumanOmni1-Quad BeadChip (Illumina, Inc., San Diego, USA). The self-report Childhood Events Questionnaire was completed for 260 GenRED 1 cases and 270 GenRED 2 cases (E. Nelson and D.Levinson, unpublished).

In the DGN study, a survey research company (Knowledge Networks, Menlo Park, CA) recruited 469 recurrent MDD cases from participants in an online survey panel that is recruited on an ongoing basis using random digit dialing of nationally-representative US households (Battle, 2014). Online screening was carried out using the Composite International Diagnostic Interview depression and alcohol and substance dependence modules (World Health Organization, 1997). Prospective cases were selected who reported two or more episodes meeting criteria for MDD but denied lifetime substance dependence. These individuals were then interviewed using the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders IV (SCID) and those not meeting the initial eligibility criteria were excluded (First, 2002). Participants were all of European ancestry and were genotyped on the Illumina HumanOmni1-Quad BeadChip (Illumina, Inc., San Diego, USA) (Battle, 2014). Childhood trauma was assessed in the DGN cases using the Childhood Events Questionnaire (E. Nelson and D. Levinson, unpublished).

The Brief Life Event Questionnaire

Stressful life events (SLEs) were assessed in RADIANT UK using the Brief Life Event Questionnaire (bLTE-Q), which is a shortened version of the List of Threatening Experiences Questionnaire (LTE-Q) (Brugha, 1985). Childbirth was added to the questionnaire to give a total of 12 items (Table S2) (Farmer, 2004). Following the LTE-Q categories, SLEs were split into those considered dependent on an individual's behaviour and those which seem independent (Table S2) (Brugha, 1985).

Table S2: Brief Life Event Questionnaire
Independent events *
Serious illness, injury or assault to subject
Serious illness, injury or assault to a close relative
Death of first-degree relative including child or spouse
Death of close family friend or second-degree relative
Something valuable lost or stolen
Dependent events ^
Separation due to marital difficulties or end of a steady relationship
Serious problem with a close friend, neighbour or relative
Subject made redundant or sacked from job
Seeking work without success for more than one month
Major financial crisis such as losing the equivalent of three months income
Problems with the police involving a court appearance
Birth of a child to the subject or their wife/partner
*Independent events are not due to the subject's behaviour.
^Dependent events may be caused by the subject's own behaviour.

Adjustment of SLEs for age and sex

Total number of SLEs was significantly associated with age ($P = 3.64 \times 10^{-8}$) and sex ($P = 0.001$), with younger individuals and females reporting more SLEs. As cases were younger than controls and contained a greater proportion of females, SLEs were adjusted for age and sex prior to the analyses. Using controls as a proxy for the general population, a linear regression of SLEs on age and sex was used to estimate their association. These regression coefficients were used to calculate the adjusted number of SLEs in the cases. Dependent and independent SLEs in cases were adjusted separately in the same manner. Prior to adjustment, the total number of SLEs predicted 16.3% of variance in case/control status using logistic regression. Dependent SLEs predicted 18.6% of variance and independent SLEs predicted 3.1% of variance. After adjustment for age and sex, the amount of variance explained decreased to 0.7%, 6.6% and 1.9% for total, dependent and independent SLEs respectively.

Supra-additive interactions

An additive model tests interaction as departure from additivity meaning that the combined effect of PRS and environment differs from the *sum* of their individual effects. This was tested using a linear regression of MDD case/ control status on the interaction term, covarying for the main effects of PRS and environment and two PCs. Models were also adjusted for PC x environment and PC x PRS interactions (Keller, 2014). No interactions were found between polygenic score and total, dependent or independent SLEs (Table S3). No significant interactions were found between PRS and CT under the additive model (Table S4).

Table S3: Additive interaction between polygenic score and SLEs

	Total SLEs			Dependent SLEs			Independent SLEs		
P_T	OR (95% C.I.)	P value	Multiple-R^2	OR (95% C.I.)	P value	Multiple-R^2	OR (95% C.I.)	P value	Multiple-R^2
0.0001	1.00 (0.99 - 1.01)	0.569	0.0001136	0.99 (0.97 - 1.01)	0.607	0.0000975	1.00 (0.98 - 1.02)	0.547	0.0001316
0.001	1.01 (0.99 - 1.02)	0.155	0.0007494	1.01 (0.99 - 1.03)	0.312	0.0003607	0.99 (0.97 - 1.02)	0.988	0.0000001
0.01	1.00 (0.99 - 1.02)	0.410	0.0002527	1.00 (0.98 - 1.02)	0.755	0.0000371	1.00 (0.98 - 1.02)	0.714	0.0000477
0.05	0.99 (0.98 - 1.01)	0.743	0.0000387	1.00 (0.98 - 1.02)	0.803	0.0000221	0.99 (0.96 - 1.01)	0.506	0.0001622
0.1	1.00 (0.98 - 1.01)	0.716	0.0000507	1.00 (0.98 - 1.02)	0.550	0.0001214	0.99 (0.97 - 1.02)	0.911	0.0000039
0.2	0.99 (0.98 - 1.01)	0.939	0.0000019	1.00 (0.98 - 1.02)	0.630	0.0000782	0.99 (0.96 - 1.01)	0.449	0.0002087
0.3	1.00 (0.98 - 1.01)	0.888	0.0000074	1.00 (0.98 - 1.02)	0.616	0.0000865	0.99 (0.97 - 1.01)	0.668	0.0000622
0.4	1.00 (0.98 - 1.01)	0.944	0.0000017	1.00 (0.98 - 1.02)	0.617	0.0000872	0.99 (0.97 - 1.01)	0.625	0.0000860
0.5	0.99 (0.98 - 1.01)	0.995	0.0000000	1.00 (0.98 - 1.02)	0.744	0.0000376	0.99 (0.97 - 1.01)	0.672	0.0000643

SLEs - stressful life events, P_T - P value threshold of the polygenic risk score, OR - odds ratio, C.I. - confidence interval

Table S4: Additive interaction between polygenic score and CT score

P_T	OR (95% C.I.)	P value	Multiple-R^2
0.0001	1.00 (0.99 - 1.00)	0.395	0.0010462
0.001	1.00 (0.99 - 1.00)	0.651	0.0003055
0.01	0.99 (0.99 - 0.99)	0.021	0.0073119
0.05	0.99 (0.99 - 0.99)	0.012	0.0093830
0.1	0.99 (0.99 - 0.99)	0.031	0.0065578
0.2	0.99 (0.99 - 1.00)	0.085	0.0044892
0.3	0.99 (0.99 - 1.00)	0.247	0.0019534
0.4	0.99 (0.99 - 1.00)	0.297	0.0015843
0.5	0.99 (0.99 - 1.00)	0.285	0.0016419

CT - childhood trauma, P_T - P value threshold of the polygenic risk score, OR - odds ratio, C.I. - confidence interval

Gene-environment correlations

Gene-environment correlations were tested using a linear regression of polygenic scores for MDD on total number of SLEs, with two principal components as covariates. This was tested in the whole sample and separately in cases and controls (Table S5). In cases, significant gene-environment correlations were found, specifically with the dependent and not independent SLEs (P= 0.001, P_T <0.001) (Table S6).

Table S5: Association between polygenic score and number of SLEs

	Whole sample			Cases			Controls		
P_T	OR (95% C.I.)	P value	Multiple-R^2	OR (95% C.I.)	P value	Multiple-R^2	OR (95% C.I.)	P value	Multiple-R^2
0.0001	1.03 (0.98 - 1.08)	0.199	0.0006102	1.04 (0.97 - 1.12)	0.213	0.0009143	1.00 (0.95 - 1.05)	0.908	0.0000124
0.001	1.06 (1.01 - 1.11)	0.014	0.0022633	1.08 (1.01 - 1.16)	0.019	0.0033593	1.01 (0.96 - 1.07)	0.503	0.0004172
0.01	1.04 (0.99 - 1.09)	0.068	0.0012501	1.06 (0.99 - 1.14)	0.089	0.0017803	1.01 (0.95 - 1.06)	0.661	0.0001798
0.05	1.03 (0.98 - 1.08)	0.210	0.0005926	1.02 (0.95 - 1.10)	0.454	0.0003474	1.01 (0.96 - 1.07)	0.484	0.0004593
0.1	1.06 (1.01 - 1.12)	0.011	0.0024902	1.08 (1.00 - 1.16)	0.036	0.0027897	1.02 (0.96 - 1.07)	0.448	0.0005537
0.2	1.05 (1.00 - 1.10)	0.052	0.0014365	1.05 (0.97 - 1.13)	0.162	0.0012461	1.02 (0.96 - 1.08)	0.410	0.0006289
0.3	1.05 (1.00 - 1.10)	0.038	0.0015548	1.06 (0.98 - 1.14)	0.114	0.0015979	1.01 (0.96 - 1.07)	0.562	0.0002994
0.4	1.05 (1.00 - 1.10)	0.046	0.0015313	1.05 (0.98 - 1.14)	0.135	0.0013779	1.02 (0.96 - 1.07)	0.442	0.0005551
0.5	1.05 (1.00 - 1.11)	0.033	0.0016629	1.06 (0.98 - 1.14)	0.122	0.0014475	1.02 (0.97 - 1.08)	0.383	0.0007198

SLEs - stressful life events, P_T - P value threshold of the polygenic risk score, OR - odds ratio, C.I. - confidence interval

Table S6: Association between polygenic score and dependent or independent SLEs in cases

	Dependent SLEs			Independent SLEs		
P_T	OR (95% C.I.)	P value	Multiple-R^2	OR (95% C.I.)	P value	Multiple-R^2
0.0001	1.03 (0.98 - 1.08)	0.236	0.0008901	1.01 (0.97 - 1.05)	0.541	0.0002301
0.001	1.08 (1.03 - 1.14)	0.001	0.0062337	1.00 (0.96 - 1.04)	0.880	0.0000136
0.01	1.04 (0.99 - 1.09)	0.098	0.0016945	1.01 (0.97 - 1.06)	0.387	0.0004716
0.05	1.03 (0.98 - 1.08)	0.235	0.0009115	0.99 (0.95 - 1.04)	0.864	0.0000195
0.1	1.07 (1.01 - 1.13)	0.011	0.0040440	1.01 (0.96 - 1.05)	0.600	0.0001718
0.2	1.06 (1.00 - 1.12)	0.026	0.0031548	0.99 (0.94 - 1.03)	0.776	0.0000517
0.3	1.06 (1.00 - 1.12)	0.023	0.0032732	0.99 (0.95 - 1.04)	0.979	0.0000005
0.4	1.06 (1.00 - 1.11)	0.030	0.0029591	0.99 (0.95 - 1.04)	0.935	0.0000045
0.5	1.06 (1.00 - 1.11)	0.031	0.0028774	1.00 (0.95 - 1.04)	0.990	0.0000001

SLEs - stressful life events, P_T - P value threshold of the polygenic risk score, OR - odds ratio, C.I. - confidence interval

No significant gene-environment correlations were found between PRS and CT score in the total sample, in depressed cases or controls (Table S7).

Table S7: Association between polygenic score and childhood trauma score

	Whole sample			Cases			Controls		
P_T	OR (95% C.I.)	P value	Multiple-R^2	OR (95% C.I.)	P value	Multiple-R^2	OR (95% C.I.)	P value	Multiple-R^2
0.0001	0.33 (0.09 - 1.17)	0.088	0.0005096	0.30 (0.04 - 2.27)	0.244	0.0057056	0.46 (0.15 - 1.39)	0.163	0.0069638
0.001	0.89 (0.25 - 3.14)	0.856	0.0000568	0.77 (0.10 - 6.02)	0.809	0.0002435	1.03 (0.35 - 3.03)	0.950	0.0000146
0.01	0.79 (0.22 - 2.81)	0.727	0.0002347	0.08 (0.01 - 0.68)	0.020	0.0220869	3.50 (1.23 - 9.91)	0.018	0.0204857
0.05	1.00 (0.28 - 3.53)	0.995	0.0000000	0.12 (0.01 - 0.98)	0.048	0.0163175	3.47 (1.20 - 10.01)	0.021	0.0195131
0.1	1.01 (0.28 - 3.62)	0.974	0.0000016	0.09 (0.01 - 0.85)	0.033	0.0184389	0.88 (0.86 - 6.80)	0.100	0.0104711
0.2	0.90 (0.25 - 3.21)	0.868	0.0000499	0.11 (0.01 - 0.96)	0.047	0.0165549	0.75 (0.73 - 6.12)	0.166	0.0072132
0.3	1.46 (0.41 - 5.20)	0.566	0.0006729	0.31 (0.03 - 2.78)	0.298	0.0045990	1.71 (0.60 - 4.91)	0.317	0.0038272
0.4	1.69 (0.47 - 6.02)	0.420	0.0012775	0.44 (0.04 - 3.97)	0.471	0.0022266	1.65 (0.57 - 4.76)	0.342	0.0033183
0.5	1.76 (0.49 - 6.28)	0.376	0.0014894	0.47 (0.05 - 4.30)	0.503	0.0018882	1.72 (0.60 - 4.90)	0.312	0.0038671

P_T - P value threshold of the polygenic risk score, OR - odds ratio, C.I. - confidence interval

Mood at Interview

Mood at the time of completion of the bLTE-Q was assessed using the Beck Depression Inventory (BDI) (Beck, 1996). Data were not available on the DeNT depression cases, leaving a subset of 1254 cases. 26.2% of cases had a score of 29 or more on the BDI and were classified as severely depressed at interview. Number of SLEs was investigated in these individuals, to test whether low mood at interview was associated with a recall bias for negative events. Cases from the GENDEP study were excluded from these analyses because they were asked to report on SLEs which occurred in the 6 months prior to the clinical trial, rather than reporting retrospectively on their worst episode of depression. Individuals who were severely depressed at the time of completion of the bLTE-Q (n=286) retrospectively reported a mean of 1.93 SLEs (s.d. 1.64) in the 6 months prior to their worst episode of depression, which was significantly higher than the mean number of 1.46 (s.d. 1.37) reported by other cases (n=889) ($P = 5.09 \times 10^{-6}$). Analysis of gene-environment correlations excluding cases who were severely depressed at interview (n=889 remaining), no longer showed any significant associations between polygenic scores and dependent SLEs (Table S8), although effects were in the same direction as in the total cases (Table S6). The lack of significance may be caused by a loss in power due to removal of half the cases rather than an association between low mood at interview and reporting of SLEs.

Table S8: Association between polygenic score and dependent SLEs in cases, excluding those severely depressed at interview

P_T	OR (95% C.I.)	P value	Multiple-R^2
0.0001	1.02 (0.95 - 1.08)	0.495	0.0005121
0.001	1.07 (1.01 - 1.15)	0.017	0.0061994
0.01	1.00 (0.94 - 1.07)	0.857	0.0000377
0.05	1.02 (0.96 - 1.09)	0.398	0.0008279
0.1	1.04 (0.98 - 1.11)	0.173	0.0021377
0.2	1.03 (0.96 - 1.10)	0.355	0.0009858
0.3	1.03 (0.96 - 1.10)	0.305	0.0011947
0.4	1.03 (0.96 - 1.10)	0.351	0.0009694
0.5	1.03 (0.96 - 1.10)	0.335	0.0010511

SLEs - stressful life events, P_T - P value threshold of the polygenic risk score, OR - odds ratio, C.I. - confidence interval

In the childhood trauma sample, individuals who were severely depressed at the time of interview (n=50) retrospectively reported a mean CT score of 50.56 (s.d. 19.81), which was significantly higher than the mean CT score of 42.70 (s.d. 15.09) reported by cases who were not severely depressed at interview (n=122) (P = 0.026). However, interactions between PRS and CT were still significant after cases severely depressed at interview were excluded from the analysis

(Table S9), suggesting that the interaction was not caused by a recall bias for negative events. This finding is consistent with two previous reports in the RADIANT UK sample, which indicated no evidence for recall bias due to low mood at interview (Fisher, 2012, Fisher, 2013).

Table S9: Multiplicative interaction between polygenic score and CT score, excluding cases severely depressed at interview

P_T	OR (95% C.I.)	P value	Nagelkerke's pseudo-R^2
0.0001	1.01 (0.98 - 1.03)	0.297	0.0035151
0.001	1.01 (0.99 - 1.03)	0.244	0.0043309
0.01	0.96 (0.94 - 0.98)	0.005	0.0257837
0.05	0.96 (0.94 - 0.98)	0.003	0.0294238
0.1	0.96 (0.94 - 0.99)	0.011	0.0191146
0.2	0.97 (0.94 - 0.99)	0.024	0.0168329
0.3	0.97 (0.95 - 1.00)	0.093	0.0092483
0.4	0.98 (0.95 - 1.00)	0.165	0.0062283
0.5	0.98 (0.95 - 1.00)	0.133	0.0072829

CT - childhood trauma, P_T - P value threshold of the polygenic risk score, OR - odds ratio, C.I. - confidence interval

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4. Reproductive fitness and genetic risk of psychiatric disorders in the general population

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Reproductive fitness and genetic risk of psychiatric disorders in the general population

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The persistence of common, heritable psychiatric disorders that reduce reproductive fitness is an evolutionary paradox. Here, we investigate the selection pressures on sequence variants that predispose to schizophrenia, autism, bipolar disorder, major depression and attention deficit hyperactivity disorder (ADHD) using genomic data from 150,656 Icelanders, excluding those diagnosed with these psychiatric diseases. Polygenic risk of autism and ADHD is associated with number of children. Higher polygenic risk of autism is associated with fewer children and older age at first child whereas higher polygenic risk of ADHD is associated with having more children. We find no evidence for a selective advantage of a high polygenic risk of schizophrenia or bipolar disorder. Rare copy-number variants conferring moderate to high risk of psychiatric illness are associated with having fewer children and are under stronger negative selection pressure than common sequence variants.

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Psychiatric disorders present a paradox which has long puzzled researchers. Mental illness usually begins in early reproductive age and those affected have fewer children than those unaffected by severe mental illness[1,2]. This, along with the substantial heritability and prevalence of psychiatric disorders, raises the question of why variants that increase the risk of such diseases have not been purged from the gene pool[3,4]. The reasons by which susceptibility alleles for mental disorders persist in the population remain elusive, but three key mechanisms have been proposed. Mutation-selection balance postulates that selection against deleterious sequence variants is balanced by the continuous occurrence of new mutations[2,3,5]. Balancing selection suggests that variants that predispose to psychiatric disorders may be beneficial under some circumstances, compensating for the deleterious effects attributable to these disorders[2,3,5]. A third possibility is an accumulation of common variants with effects on psychiatric disorders that are individually too weak to be effectively targeted by negative selection in most human populations[6].

Rare recurrent copy-number variants (CNVs) with large effects have been associated with schizophrenia, autism and bipolar disorder in a subset of patients[7,8]. Two such CNVs have been associated with decreased reproductive fitness in carriers in the general population, with male fitness being particularly affected[9]. The persistence of these CNVs in the gene pool is consistent with mutation-selection balance. However, psychiatric disorders also have a polygenic component, arising from the combined effect of many common risk variants, each with small effect[4]. Polygenic risk scoring is a method of summarizing an individual's genetic liability for a trait, by weighting alleles according to effect sizes estimated in genome-wide association studies (GWAS)[10]. These effects are summed together into a polygenic risk score (PRS), that reflects the cumulative impact of many common variants on a phenotype.

Association between PRS for psychiatric disorders and having fewer children in undiagnosed individuals from the general population would indicate that common risk variants are subject to negative selection. Association between PRS and having more children in individuals unaffected by psychiatric disorders would support balancing selection. Lack of association between PRS and number of children would indicate that there is either no selection affecting these variants or weak selection pressure which currently cannot be detected. Here, we test these hypotheses using data from a sample of 150,656 Icelanders representing approximately half of the population. CNVs implicated in autism and schizophrenia are also tested for association with number of children in carriers in the general population, to compare the selection pressures on the common and rare components of the genetic basis of psychiatric disorders. Finally, we determine whether PRS for psychiatric disorders are associated with age at first child in the Icelandic population, to further dissect the link between parental age and risk of psychiatric disorders[11,12].

Our data show that polygenic risk for autism is associated with fewer children and later age at first child, while polygenic risk for ADHD is associated with having more children. We find no evidence for a selective advantage of high polygenic risk for schizophrenia or bipolar disorder. Rare CNVs conferring moderate to high risk of psychiatric illness are associated with having fewer children and are under stronger negative selection pressure than common genetic variants.

Results

PRS and psychiatric disorders. PRS for five psychiatric disorders were generated for each genotyped individual using the results of independent GWAS on schizophrenia, bipolar disorder, autism, attention deficit hyperactivity disorder (ADHD) and major depression, available online from the Psychiatric Genomics Consortium (https://pgc.unc.edu/)[13–17]. We first tested the predictive power of each PRS for their corresponding disorder within the Icelandic sample. All scores were significantly associated with their matching disorder (Fig. 1). The maximum variance explained was 6.4% for schizophrenia ($P = 2.1 \times 10^{-109}$). Other PRS explained up to 0.6% of the variance for the corresponding diseases (Fig. 1).

PRS and number of children. We used a subset of 93,720 genotyped subjects, aged at least 45 years and without a diagnosis of a psychiatric disorder, to test the association of each PRS with the number of children born to each individual. There was a negative association between the PRS for autism and number of children ($\beta = -0.25$, $P = 0.002$) (Table 1), while higher PRS for ADHD was associated with having more children ($\beta = 0.15$, $P = 0.002$) (Table 1). Other PRS were not associated with number of children after Bonferroni correction for multiple tests (Table 1, Supplementary Figs 1–5). The quadratic effects of the PRS were also examined, to test whether very high or low PRS may be associated with number of children, but these results were non-significant. Furthermore, there were no associations between variance in number of children and deciles of PRS in the total sample, males or females, after multiple testing correction (Supplementary Figs 6–10).

Neuropsychiatric CNVs and number of children. In a subset of patients with schizophrenia or autism, CNVs are likely to be the strongest individual factors contributing to the pathogenesis of the disorder. Eleven CNVs conferring risk of schizophrenia or autism ('neuropsychiatric CNVs') were tested for association with number of children, excluding individuals with autism, schizophrenia, bipolar disorder and intellectual disability[7,8]. Collectively, carriers of a neuropsychiatric CNV ($N = 469$) had significantly fewer children than non-carriers ($N = 91{,}987$) ($\beta = -0.279$, $P = 0.0001$), with a greater reduction in males than females (Table 2). After correction for multiple comparisons, the 16p11.2 deletion was individually associated with having fewer children (Table 2).

Selection pressures on PRS versus neuropsychiatric CNVs. Individuals in the top 1% of schizophrenia PRS have an odds ratio of 9.5 (95% CI 6.8 − 13.3) of developing the disorder. While this risk is of a magnitude similar to that conferred by a single neuropsychiatric CNV, individuals with high PRS of schizophrenia have the same number of children as the rest of the population ($\beta = 0.054$, $P = 0.29$). However, they have a greater variance in number of children ($\beta = 1.112$, $P = 0.02$). The same comparison was performed for individuals in the top 1% of PRS for autism and bipolar disorder. The difference in number of children was not statistically significant in the former ($\beta = -0.085$, $P = 0.095$, OR for autism = 2.4) while those in the top 1% of bipolar disorder PRS were found to have fewer children than the rest of the population ($\beta = -0.148$, $P = 0.0037$, OR for bipolar disorder = 1.6).

PRS and age at first child. PRS for autism is associated with later age at first child in the total sample ($\beta = 0.97$, $P = 0.0004$) (Table 3). PRS for ADHD is associated with younger age at first child for both sexes ($\beta = -0.59$, $P = 0.0003$) and PRS for MDD is associated with younger age at first child in females ($\beta = -0.56$, $P = 0.00009$) (Table 3). Quadratic effects of PRS were not found to be associated with age at first child.

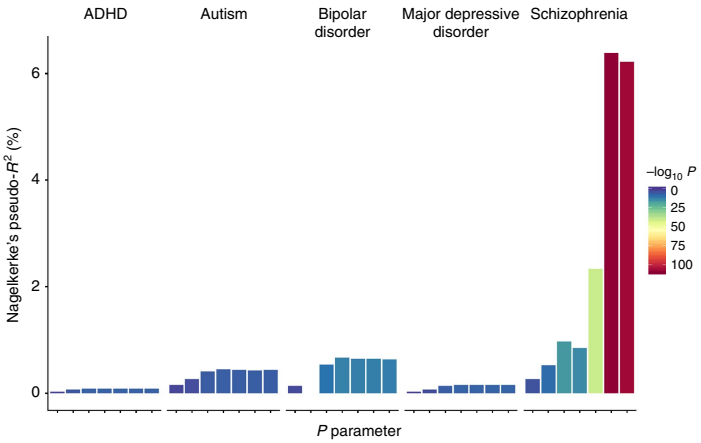

Figure 1 | Polygenic risk scores for psychiatric disorders predict their corresponding disorder in the general population of Iceland. The *x* axis shows the seven *P*-value parameters (0.001, 0.003, 0.01, 0.03, 0.1, 0.3 and 1.0) used to weight SNPs from the discovery GWAS plotted left to right. The *y* axis indicates the Nagelkerke's pseudo-R^2 measure of variance explained.

Table 1 | Association between polygenic risk scores and number of children.

Polygenic score	Total population		Males		Females	
	P value	Beta (CI)	*P* value	Beta (CI)	*P* value	Beta (CI)
ADHD	0.002	0.15 (0.05, 0.25)	0.170	0.09 (−0.04, 0.24)	0.002	0.20 (0.07, 0.33)
Autism	0.002	−0.25 (−0.41, −0.09)	0.003	−0.36 (−0.59, −0.12)	0.130	−0.16 (−0.38, 0.05)
Bipolar disorder	0.740	−0.005 (−0.03, 0.02)	0.500	0.01 (−0.02, −0.05)	0.310	−0.02 (−0.05, 0.01)
Major depression	0.170	0.04 (−0.01, 0.11)	0.650	0.02 (−0.07, 0.12)	0.094	0.07 (−0.01, 0.16)
Schizophrenia	0.160	0.006 (−0.002, 0.01)	0.530	0.004 (−0.008, 0.02)	0.170	0.007 (−0.003, 0.02)

In total, ten tests on number of children were performed; thus the significance threshold is $P<0.005$.
CI—95% confidence interval

Discussion

Here we have investigated selection pressures acting on sequence variants conferring risk of psychiatric disorders in recent generations of Icelanders by testing whether PRSs and neuropsychiatric CNVs are associated with number of children in a large population sample that excludes patients diagnosed with the psychiatric diseases. PRS for autism was found to be associated with having fewer children, indicating that common variants that are thousands of years old are currently subject to weak negative selection pressure.

Balancing selection as an explanation for the persistence of these variants means that variants that increase the risk of psychiatric disorders may persist if their negative effects on fitness in affected carriers are offset by benefits in individuals who carry the variants but do not develop the disorders[3]. PRS for schizophrenia and bipolar disorder have been found to predict creativity in the general population of Iceland[18]. However, creative individuals in Iceland have fewer children than population controls[18]. Based on these results, along with the lack of association between these PRS and number of children found here, we conclude that there is no evidence for a selective advantage that maintains common variants associated with schizophrenia or bipolar disorder. While greater PRS for ADHD is associated with having more children in the Icelandic population, we do not interpret this as support for balancing selection. This is because individuals with ADHD have more children than average in Iceland and in other populations[19].

We recognize that a Bonferroni correction may be over-conservative for this analysis, as one and the same variant may predispose to several psychiatric disorders, meaning that the PRS are not totally independent[20,21]. Furthermore, several of these PRS currently only explain a small amount of variance in risk of the disorders themselves (Fig. 1) and therefore may be underpowered to detect associations with number of children, even in this large population sample. As GWAS with greater statistical power are conducted on psychiatric disorders and their polygenic component can be estimated with greater accuracy, other associations with reproductive fitness may be uncovered. One limitation of the current study is that selection pressures in the modern environment may not be the same as those that acted on our ancestors. For example, in recent times there have been far-reaching cultural changes in factors such as education and the use of contraception, which have led to a postponement of having a first child and a reduction in number of children in many human populations. In our data, subjects diagnosed with psychiatric disorders were excluded. However, some unidentified patients in the sample could obscure evidence for balancing selection.

Here, neuropsychiatric CNVs implicated in schizophrenia, autism and bipolar disorder are associated with fewer children, particularly among males. These CNVs are rare and generally have large effects[7,8]. Several have been associated with lower IQ, cognitive deficits and other physical abnormalities, that may have a negative impact on reproductive fitness in population controls

Table 2 | Association between neuropsychiatric CNVs and number of children.

CNV	Odds ratio (autism/schizophrenia)	Carriers (male/female)	Non-carriers (male/female)	Beta (male/female)	*P* value (male/female)
All CNVs		201/268	42,826/49,161	−0.405/−0.184	0.00028/0.050
Autism CNVs		37/60	42,990/49,369	−1.263/−0.481	9.4E−07/0.014
Schizophrenia CNVs		189/256	42,838/49,173	−0.266/−0.115	0.021/0.23
16p11.2 del	inf/NA	12/12	43,015/49,417	−2.534/−1.59	1.8E−08/0.00025
1q21.1 del	NA/8.35	11/15	43,016/49,414	−0.741/−0.98	0.12/0.012
22q11.21 del	NA/inf	3	92,453	−1.421	0.11
16p11.2 dup	4.1/11.52	39	92,417	−0.326	0.19
15q11.2—13.1 dup	inf/13.20	4	92,452	−0.926	0.23
7q11.23 (WBS) dup	NA/11.35	3	92,453	−1.150	0.23
15q13.3 all del	inf/7.52	19	92,437	−0.343	0.34
16p13.1 dup	NA/2.30	103	92,353	−0.128	0.41
2p16.1 (NRXN1) del	5.6/9.01	11	92,445	−0.353	0.45
15q11.2 all del	NA/2.15	192	92,264	−0.058	0.61
1q21.1 dup	NA/3.45	38	92,418	0.049	0.85

Results are shown in males and females separately when there is a significant ($P<0.05$) association in either group. Counting all models fitted, 28 tests were performed; thus the significance threshold is $P<0.0017$. Odds ratios for autism and schizophrenia are from the literature[7,8].

Table 3 | Association between polygenic risk scores and age at first child.

Polygenic score	Total sample		Males		Females	
	P value	Beta (CI)	*P* value	Beta (CI)	*P* value	Beta (CI)
ADHD	0.0003	−0.59 (−0.92, −0.26)	0.07	−0.48 (−0.99, −0.03)	0.0005	−0.71 (−1.12, −0.31)
Autism	0.0004	0.97 (0.43, 1.51)	0.03	0.91 (0.08, 1.76)	0.001	1.10 (0.45, 1.77)
Bipolar disorder	0.005	0.14 (0.04, 0.23)	0.18	0.10 (−0.04, 0.25)	0.007	0.16 (0.04 − 0.28)
Major depression	0.006	−0.31 (−0.54, −0.09)	0.71	−0.06 (−0.42, 0.28)	9.00E−05	−0.56 (−0.84, −0.28)
Schizophrenia	0.07	−0.02 (−0.05, 0.003)	0.81	−0.005 (−0.05, 0.04)	0.02	−0.04 (−0.07, −0.01)

In total, ten tests on age at first child were performed; thus the significance threshold is $P<0.005$.
CI—95% confidence interval

who have not been diagnosed with psychotic or neurodevelopmental disorders[9,22]. De *novo* CNVs have been implicated in cases of schizophrenia, autism and bipolar disorder which supports mutation-selection balance. A previous study has also shown that there is a strong selection against schizophrenia-associated CNVs, such that these variants persist in the population for only a few generations after they arise[23].

Finally, age at first child is of particular interest as Western society has experienced a rapid postponement of parenthood[24], which has been associated with reduction in polygenic score for educational attainment[25]. Delayed fatherhood has been linked to risk of psychiatric disorders, widely assumed to be caused by the accumulation of *de novo* mutations in the spermatogonial stem cells of older males[26,27]. However, recent population genetic modelling suggests that these mutations are unlikely to explain much of the risk and a weak correlation between age at first child and genetic liability to psychiatric illness could account for the observed incidence of the disorders in the children of older fathers[11]. In accordance with this hypothesis, PRS for autism was positively associated with later age at first child in the Icelandic population, providing an alternative explanation of age-related mutations.

In summary, our results show that common sequence variants conferring risk of autism and ADHD are currently under weak selection in the general population of Iceland. However, rare CNVs that also impact cognition are under stronger selection pressure, consistent with mutation-selection balance. The hypothesis that a selective advantage accounts for the prevalence of sequence variants conferring risk of schizophrenia and bipolar disorder is unproven, but rather this empirical evidence suggests that common sequence variants largely escape selection as their individual effect sizes are weak.

Methods

Subjects. The study was approved by the National Bioethics Committee of Iceland and the Icelandic Data Protection Authority. Samples are from a population genetic biobank of 150,656 Icelanders established by deCODE genetics. Reproductive fitness was defined as the number of children born to individuals over 45 years. Subjects born before 1968 with matching genotypic data ($N=93,720$) were identified from deCODE's nation-wide genealogy database. This contains information on year of birth, county of birth and numbers of children of Icelanders. Diagnoses of schizophrenia and bipolar disorder were assigned according to Research Diagnostic Criteria (RDC)[28] through the use of the Schedule for Affective Disorders and Schizophrenia Lifetime Version (SADS-L)[29]. ADHD subjects were recruited from outpatient pediatric, child and adult psychiatry clinics in Iceland; ICD-10 diagnoses were made on the basis of standardized diagnostic assessments by experienced clinicians. Autism subjects were ascertained through the State Diagnostic Counseling Center and the Department of Child and Adolescent Psychiatry in Iceland and received ICD-10 diagnoses based on standardized diagnostic assessments by clinical specialists. Diagnoses of MDD were made by clinicians or based on the results of a semi-structured interview (CIDI), and were assigned according to DSM-III, ICD-9 or ICD-10 criteria. All diagnoses of recurrent depression were included (that is, mild, moderate and severe), but in the case of single episode depression mild cases were excluded. Characteristics of the sample are shown in Supplementary Table 1.

Genotyping and imputation. Genotyping was performed on Illumina HumanHap (300, 370, 610, 1 M, 2.5 M) and IlluminaOmni (670, 1 M, 2.5 M, Express) SNP arrays[9]. BeadStudio (Illumina; version 2.0) was used to call genotypes, normalize signal intensity data and establish the log R ratio and B allele frequency at every SNP. Long-range haplotype phasing was achieved using an iterative algorithm, which phases a single proband at a time, given the available phasing information on all other individuals who share a long haplotype identically by state with the

proband[30]. Given the large proportion of the Icelandic population that has been chip-typed, accurate genome-wide long-range phasing is possible for all chip-typed Icelanders. For long-range phased haplotype association analysis, the genome was then partitioned into non-overlapping fixed 0.3 cm bins. Within each bin, the haplotype diversity was consistent with the combination of all chip-typed markers in the bin. The whole genomes of 8,453 Icelanders were sequenced using Illumina technology to a mean depth of at least × 10 (median × 32). SNPs and indels were identified and genotypes called using join calling with the Genome Analysis Toolkit Haplotype Caller (GATK version 3.3.0) (ref. 31). The error rate of genotype calls made solely on the basis of next generation sequence data decreases as a function of sequencing depth. Taking advantage of the fact that all the sequenced individuals had also been chip-typed and long-range phased, information about haplotype sharing was utilized to minimize the number of such errors. Thus, the genotype call in cases where sequence reads were ambiguous would be informed by comparison with sequence reads of other individuals sharing haplotypes with the individual in question at the ambiguous site. To improve genotype quality and to phase the sequencing genotypes an iterative algorithm based on the IMPUTE HMM model[32] and using the long range phased haplotypes was employed[33]. The same principle was then used to impute the sequence variants identified in the 8,453 sequenced Icelanders into 150,656 Icelanders who had been genotyped with various Illumina SNP arrays and their genotypes phased using long-range phasing[33].

Polygenic risk scoring and CNV selection. We derived PRSs from GWAS summary results available online from the Psychiatric Genomics Consortium (https://pgc.unc.edu/) for ADHD, autism, bipolar disorder, major depression and schizophrenia[13–17]. The number of cases in these studies was 896, 3,303, 7,481, 9,240 and 35,476 respectively. The deCODE sample was not part of these analyses. To compute the PRSs we used approximately 630,000 autosomal markers from a framework set of markers used in long-range haplotype phasing. The framework markers have been selected on the basis of various quality criteria including high genotype yield, Hardy–Weinberg equilibrium and consistency of allele frequencies across different Illumina array types. We estimated the linkage disequilibrium between markers using Icelandic samples and adjusted for it using LDpred[34] a recently proposed method. PRSs were calculated with seven different settings of the P parameter (corresponding roughly to the fraction of causal markers[34]): 0.001, 0.003, 0.01, 0.03, 0.1, 0.3 and 1.0. Eleven CNVs conferring risk of schizophrenia or autism ('neuropsychiatric CNVs') were selected from the most recent review on CNVs in schizophrenia[7] and the most recent analysis of CNVs in autism[8].

Statistical analysis. PRSs were first tested for association with their corresponding psychiatric disorder in the Icelandic population ($N = 1{,}137$, 692, 806, 3,246 and 631 cases for ADHD, autism, bipolar disorder, major depression and schizophrenia respectively). This was performed using logistic regression with five principal components as covariates. Models were compared against a null model including covariates only to calculate the Nagelkerke's pseudo-R^2 measure of variance explained. A linear mixed effects model was used to test the association of number of children with PRS for the five psychiatric disorders. Number of children was regressed on the PRS of interest, covarying for year of birth, sex and interaction between the two, birth county of last child or birth county of the parent, five principal components and sibship (to account for relatedness). For each respective disorder we chose the PRS calculated with a P parameter corresponding to a fraction of causal markers of 0.3 and modelled the correlation with its respective disorder and then recalibrated the PRS to have a mean of 0 and a unit increase corresponding to a doubling of risk for the disorder. All predictors were modelled as fixed effects apart from sibship which was random. This model was compared against a null model including the covariates only. To test the quadratic effects of the PRS, a PRS squared term was added and PRS was included in the null model. Sex-specific analyses were also conducted. Individuals diagnosed with each psychiatric disorder were excluded, although it may not be possible to identify every past case in a general population sample. Age at first child was tested for association with each PRS in the same manner. To examine the relationship between variance in number of children and PRSs, PRSs were split into deciles and number of children was adjusted for all covariates. A linear regression was used to test the association between deciles of PRS and residual number of children in the total sample, males and females. Neuropsychiatric CNVs were examined for association with number of children using the following covariates: year of birth, sex and interaction between the two, birth county of last child or birth county of the parent, five principal components and the random effect of sibship. Individuals with autism, schizophrenia, bipolar disorder and intellectual disability were excluded from the CNV analyses.

Data availability. Data supporting the findings of this study are available within the article and its Supplementary Information files. Summary level data from the PGC GWAS used to calculate PRS in this study were obtained from the PGC Downloads website (https://www.med.unc.edu/pgc/results-and-downloads/).

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Author contributions

N.M., C.M.L., H.S. and K.S. were involved in study design. H.S., E.S. and K.S. were involved in cohort ascertainment, phenotypic characterization and recruitment. J.E., A.I., D.F.G., M.L.F., A.K., H.S. and G.B.W. were involved in informatics and data management. N.M., H.P., A.G. and S.Ó. carried out statistical analysis. N.M., A.H., D.F.G., H.S. and K.S. wrote the first draft of the manuscript and all authors contributed to the final version of the manuscript.

Additional information

Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

Competing interests: A.I., S.Ó, D.F.G., Ó.Ó.G., M.L.F., A.K., A.H., G.B.W., O.G., H.S. and K.S. are employees of deCODE Genetics/Amgen. The remaining authors declare no competing financial interests.

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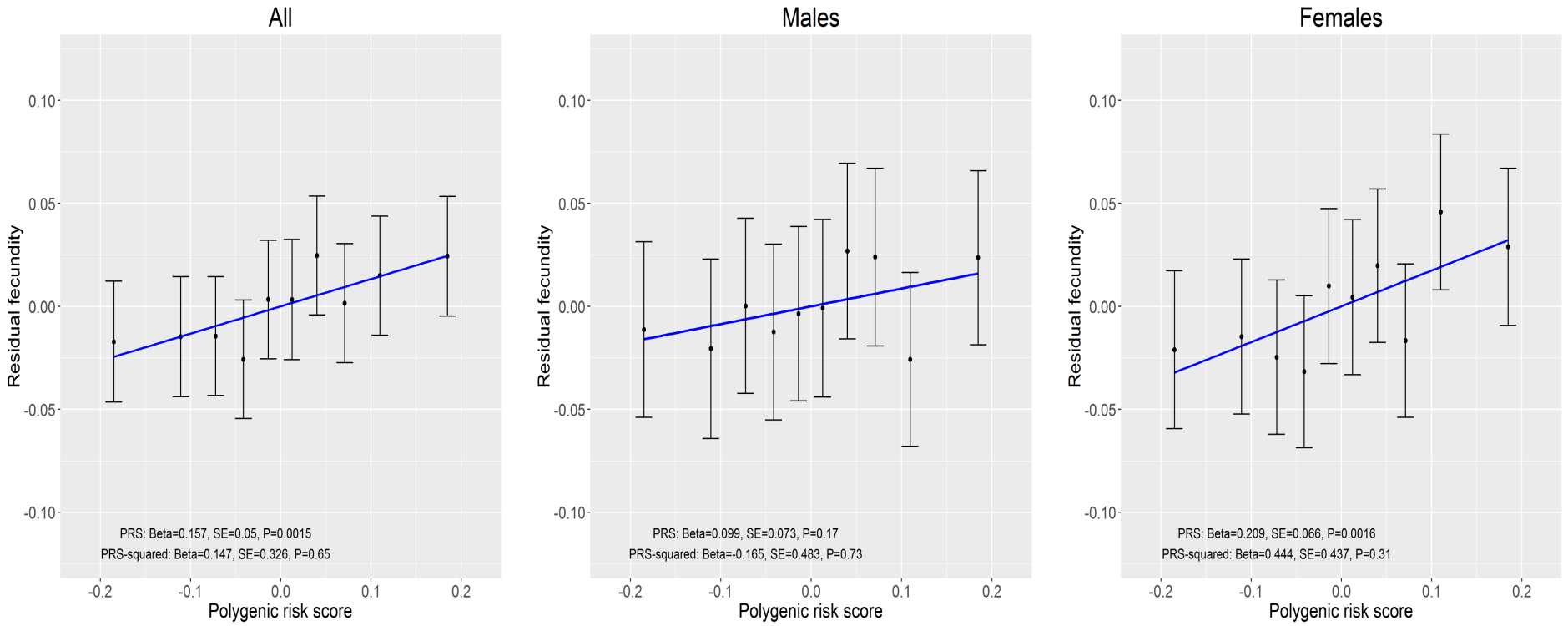
Supplementary material

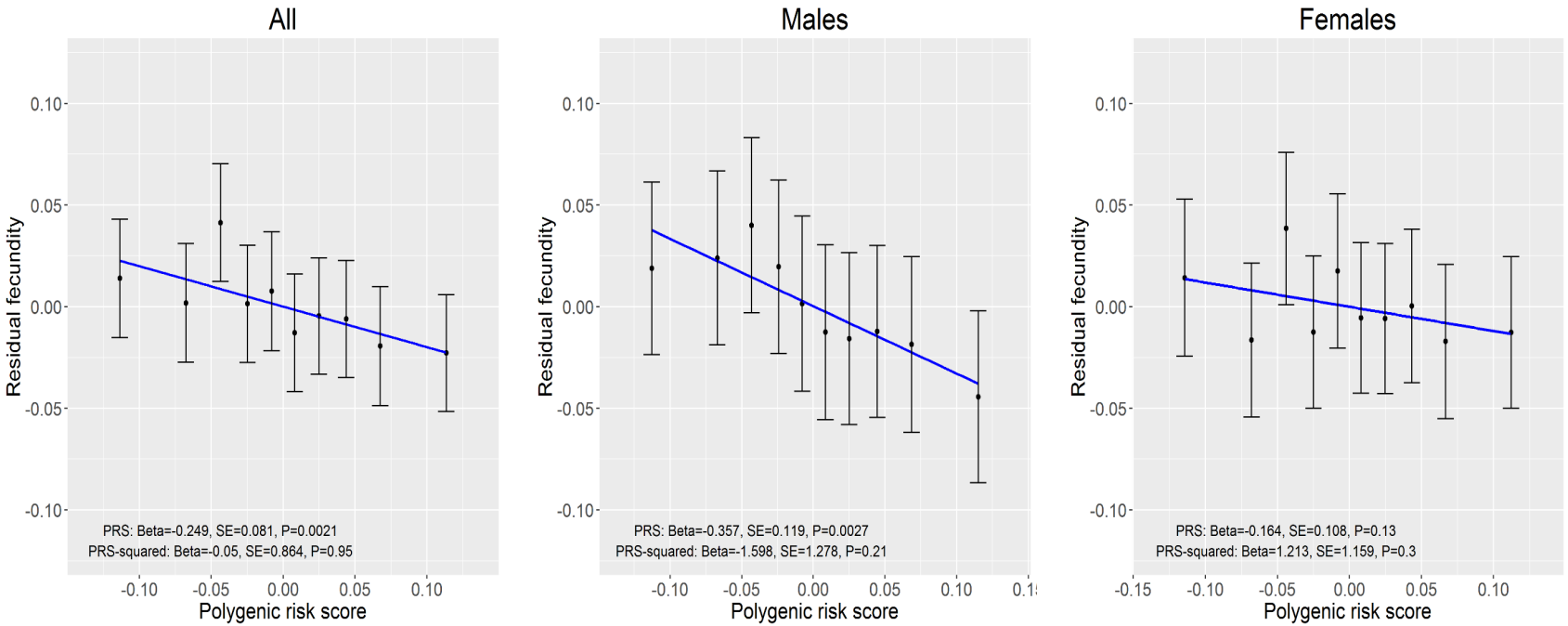
Supplementary Table 1: Sample characteristics of genotyped individuals born before 1968 (n = 93,720)	
Mean age (years) (s.d.)	68.91 (14.55)
Male (%)	43 606 (46.53)
Female (%)	50 114 (53.47)
Mean number of children (s.d.)	2.91 (1.60)
Mean age at first child (years) (s.d.)	24.09 (5.15)
Attention deficit hyperactivity disorder (%)	105 (0.11)
Autism (%)	145 (0.15)
Bipolar disorder (%)	653 (0.70)
Major depressive disorder (%)	2189 (2.33)
Schizophrenia (%)	463 (0.49)

s.d. - standard deviation

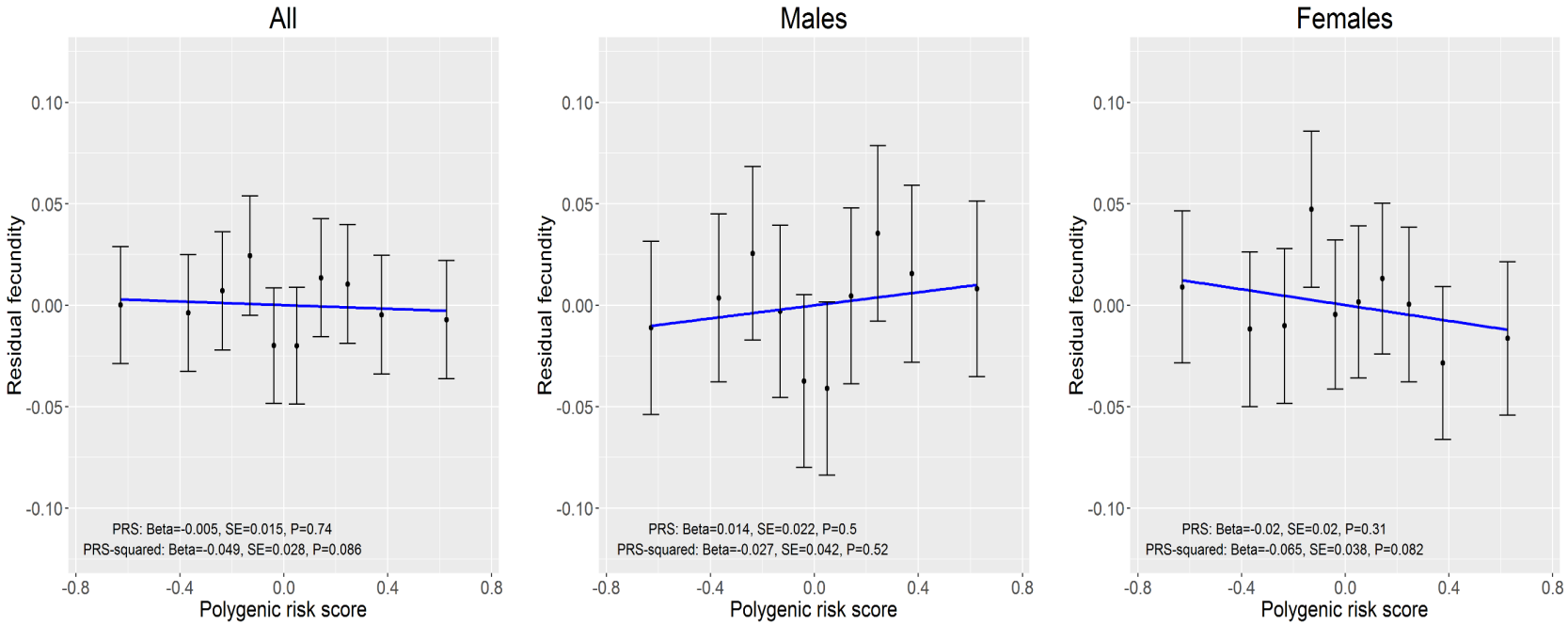


Supplementary Figure 1: ADHD polygenic risk score decile versus residual number of children in the total sample, males and females

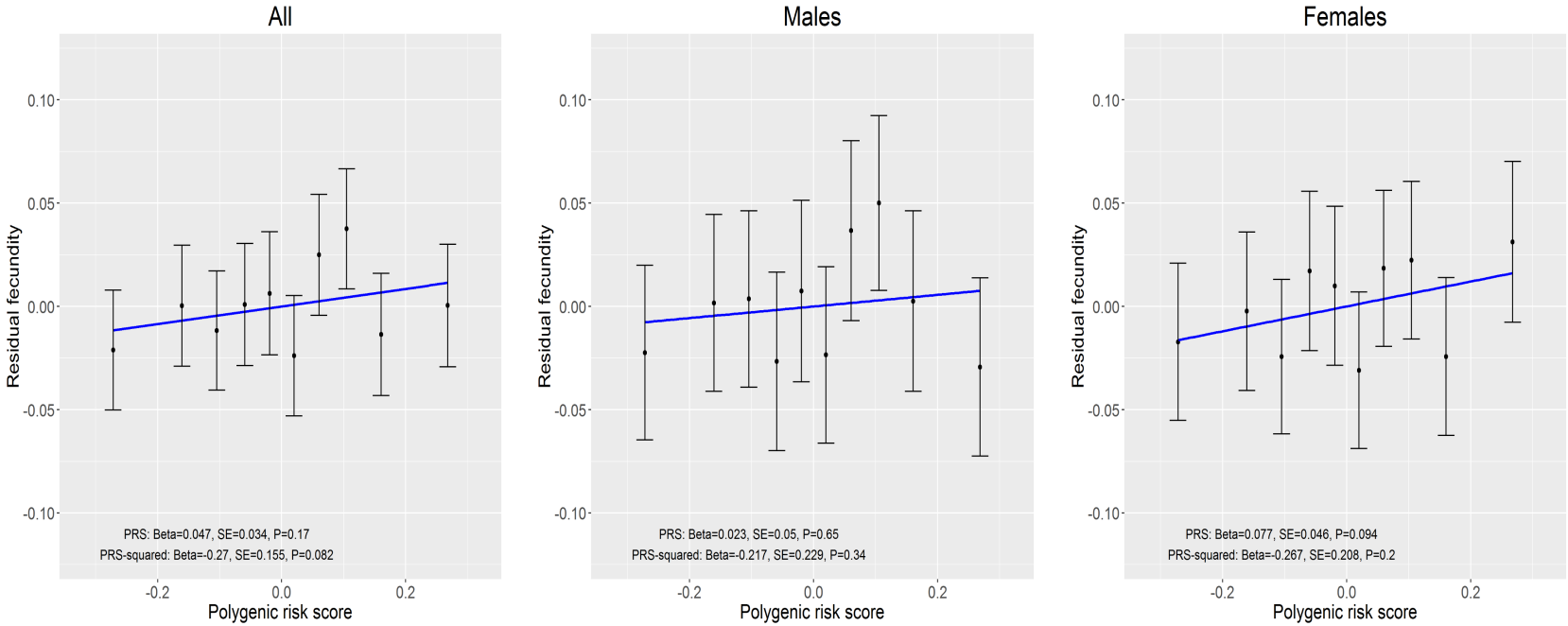
Residual fecundity is number of children adjusted for year of birth, birth county of the last child, 5 principal components and sibship as a random effect. Polygenic risk scores were calculated using *P* value parameter 0.3 and recalibrated to have a mean of 0 and a unit increase corresponding to a doubling of risk for the disorder. The x-axis shows the mean polygenic risk score per decile. Affected patients are excluded.



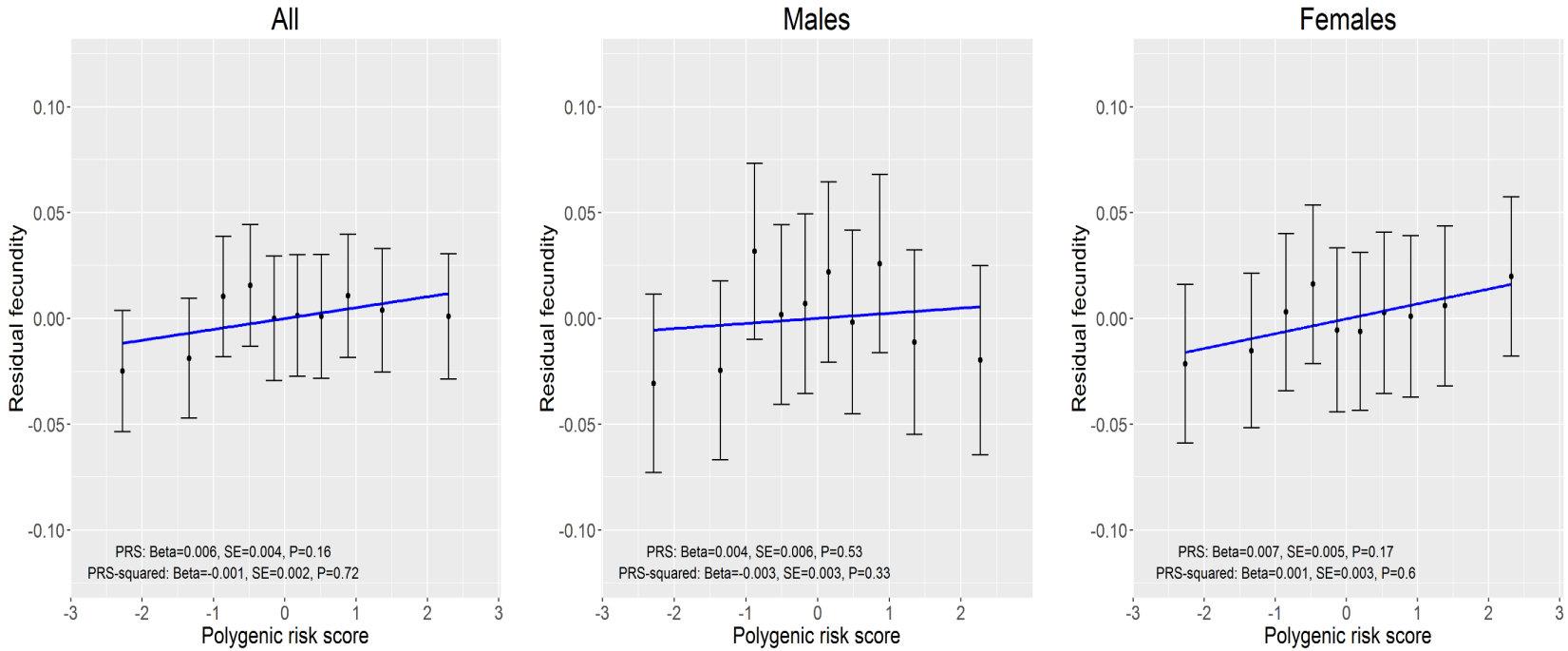
Supplementary Figure 2: Autism polygenic risk score decile versus residual number of children in the total sample, males and females
Residual fecundity is number of children adjusted for year of birth, birth county of the last child, 5 principal components and sibship as a random effect. Polygenic risk scores were calculated using *P* value parameter 0.3 and recalibrated to have a mean of 0 and a unit increase corresponding to a doubling of risk for the disorder. The x-axis shows the mean polygenic risk score per decile. Affected patients are excluded.



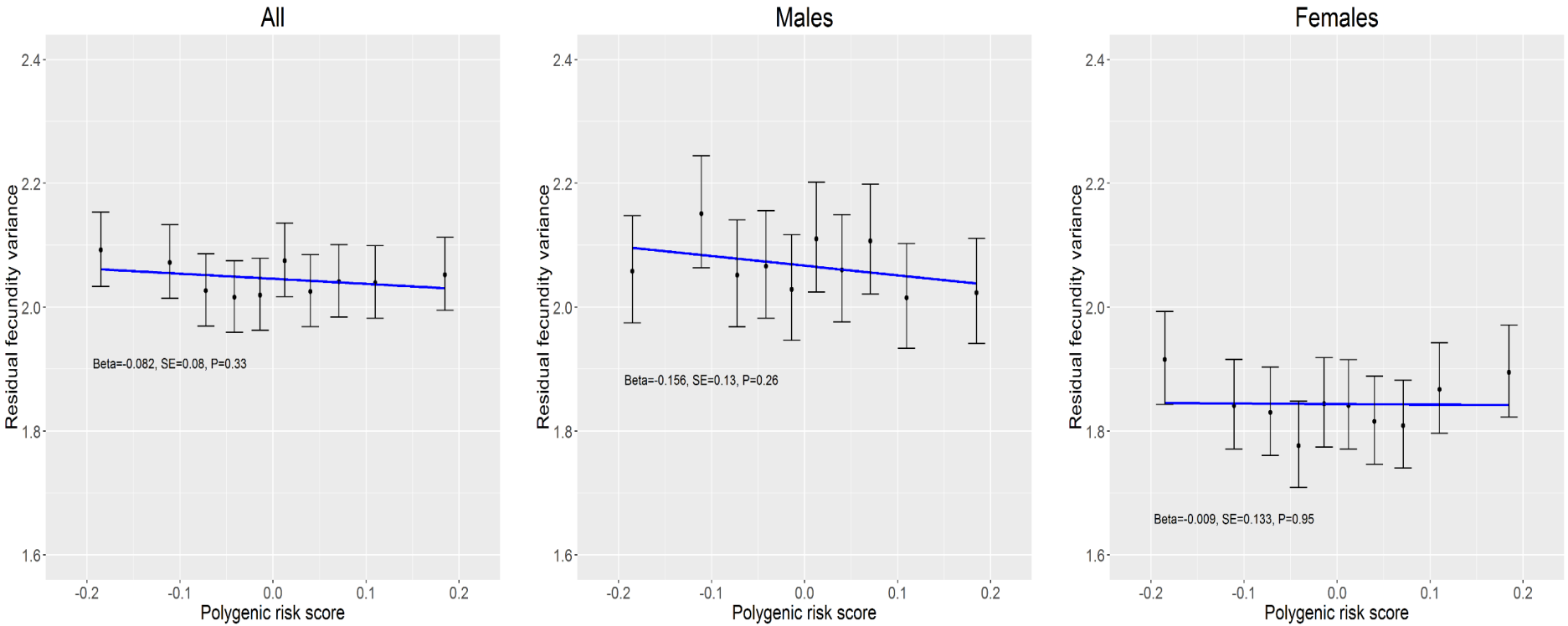
Supplementary Figure 3: Bipolar disorder polygenic risk score decile versus residual number of children in the total sample, males and females
Residual fecundity is number of children adjusted for year of birth, birth county of the last child, 5 principal components and sibship as a random effect. Polygenic risk scores were calculated using *P* value parameter 0.3 and recalibrated to have a mean of 0 and a unit increase corresponding to a doubling of risk for the disorder. The x-axis shows the mean polygenic risk score per decile. Affected patients are excluded.



Supplementary Figure 4: Major depression polygenic risk score decile versus residual number of children in the total sample, males and females
Residual fecundity is number of children adjusted for year of birth, birth county of the last child, 5 principal components and sibship as a random effect. Polygenic risk scores were calculated using *P* value parameter 0.3 and recalibrated to have a mean of 0 and a unit increase corresponding to a doubling of risk for the disorder. The x-axis shows the mean polygenic risk score per decile. Affected patients are excluded.

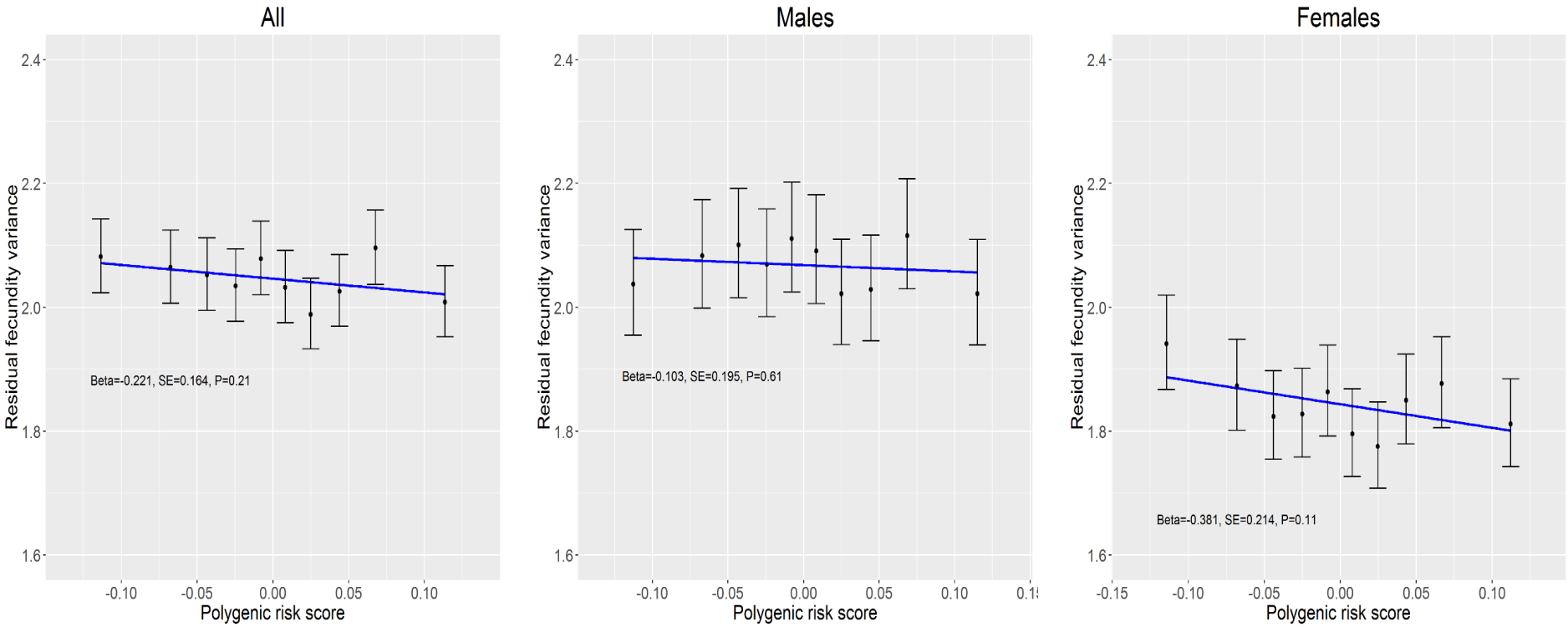


Supplementary Figure 5: Schizophrenia polygenic risk score decile versus residual number of children in the total sample, males and females
Residual fecundity is number of children adjusted for year of birth, birth county of the last child, 5 principal components and sibship as a random effect. Polygenic risk scores were calculated using *P* value parameter 0.3 and recalibrated to have a mean of 0 and a unit increase corresponding to a doubling of risk for the disorder. The x-axis shows the mean polygenic risk score per decile. Affected patients are excluded.

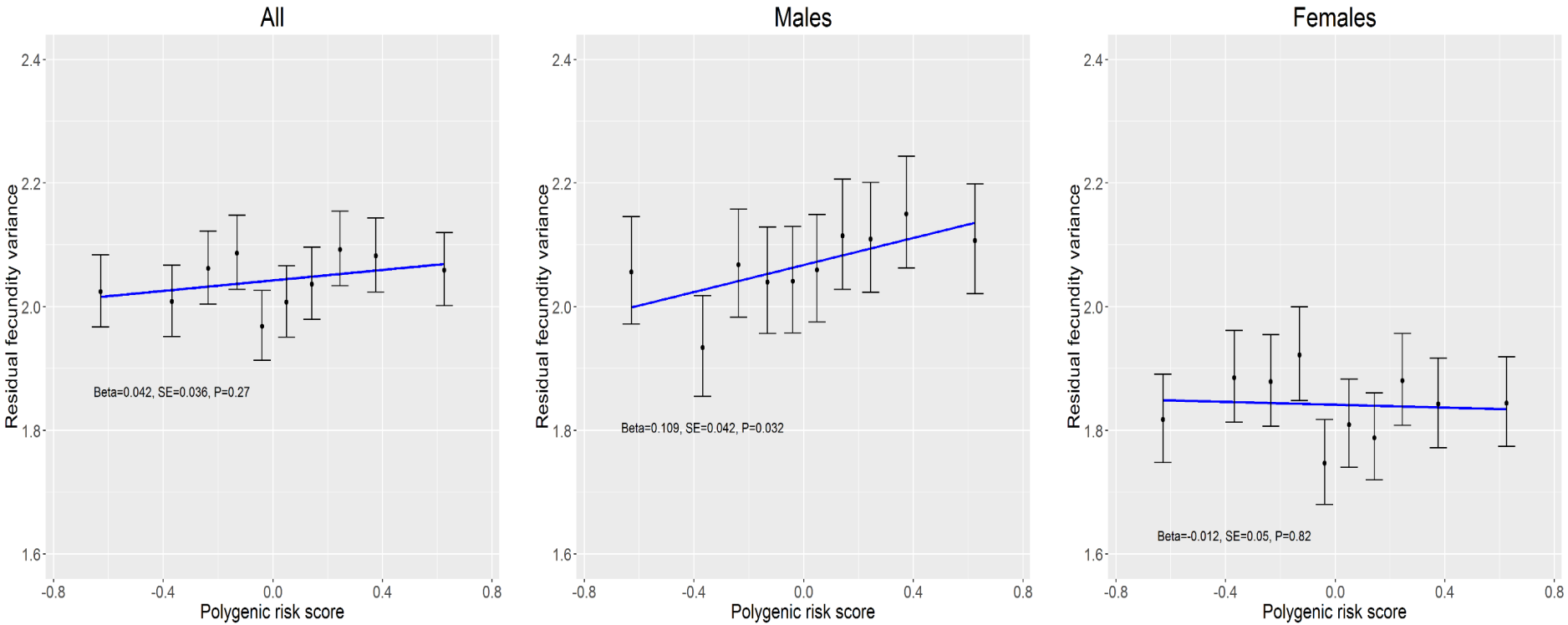


Supplementary Figure 6: ADHD polygenic risk score decile versus variance in residual number of children in the total sample, males and females

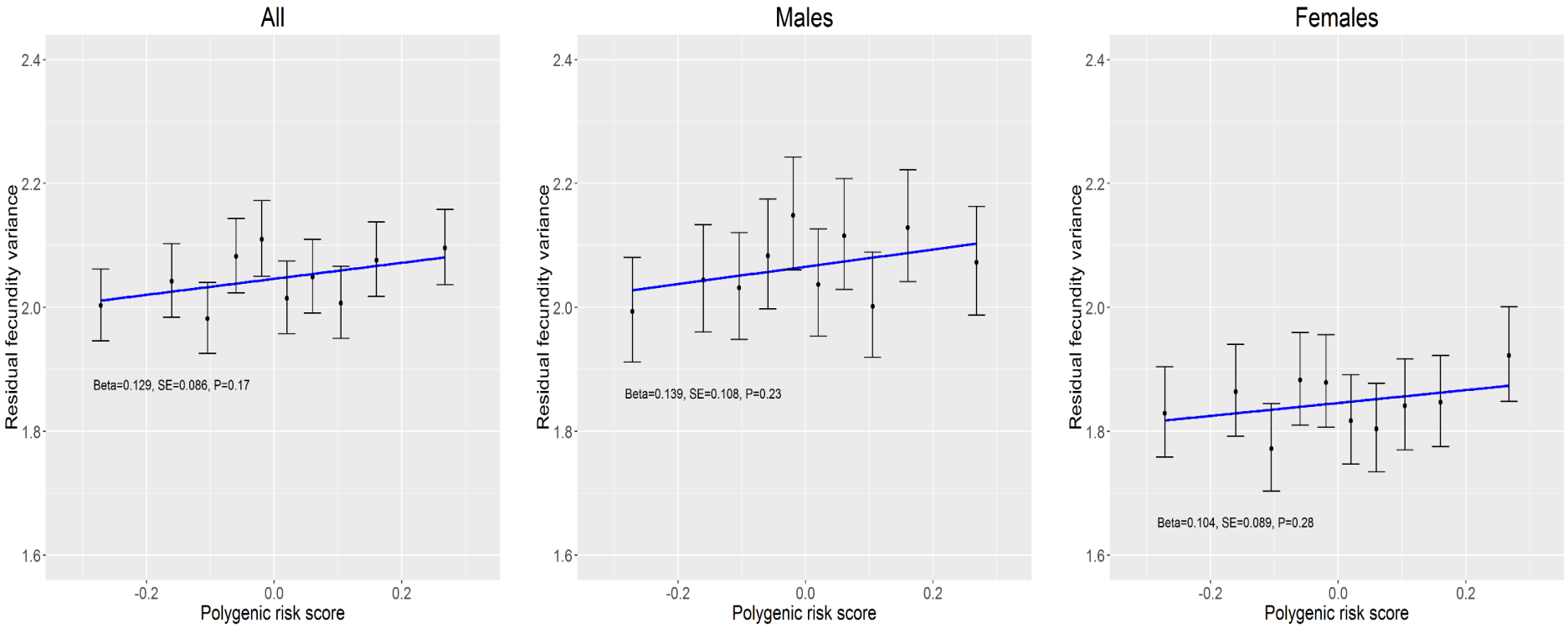
Residual fecundity is number of children adjusted for year of birth, birth county of the last child, 5 principal components and sibship as a random effect. Polygenic risk scores were calculated using *P* value parameter 0.3 and recalibrated to have a mean of 0 and a unit increase corresponding to a doubling of risk for the disorder. The x-axis shows the mean polygenic risk score per decile. Affected patients are excluded.



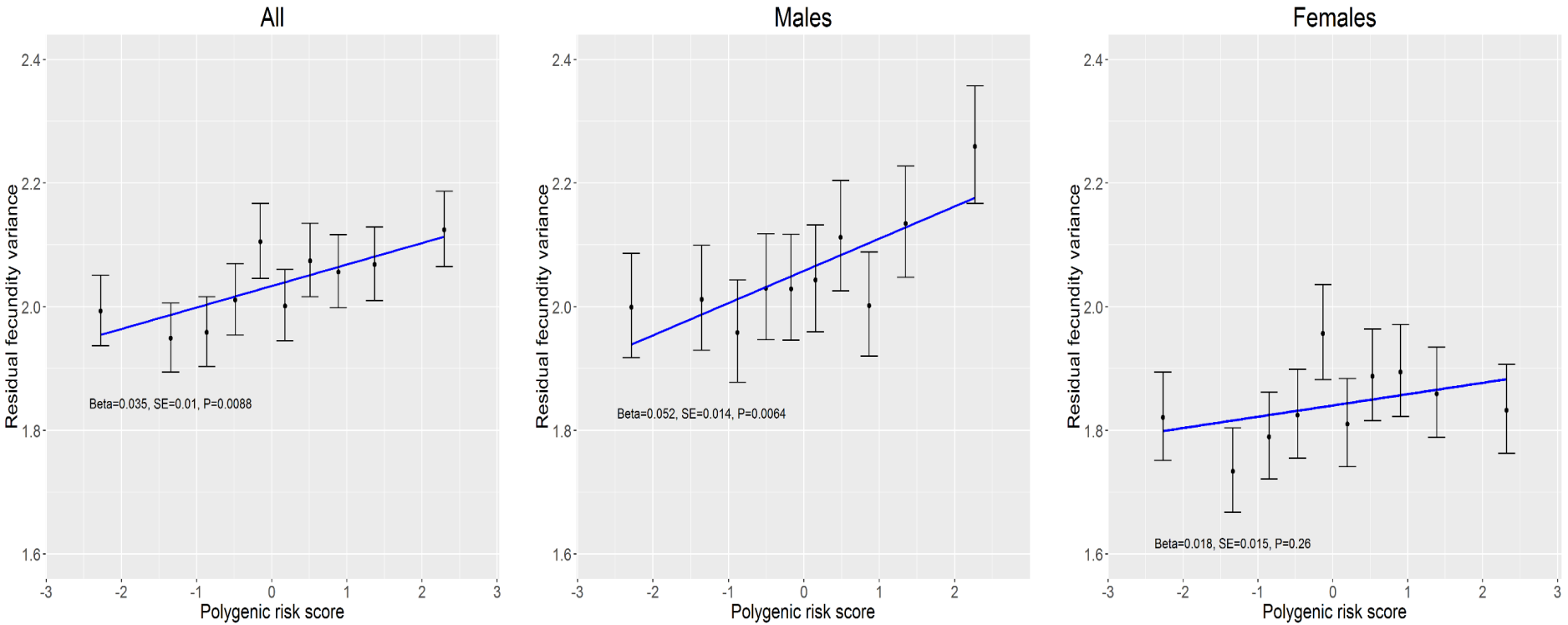
Supplementary Figure 7: Autism polygenic risk score decile versus variance in residual number of children in the total sample, males and females
Residual fecundity is number of children adjusted for year of birth, birth county of the last child, 5 principal components and sibship as a random effect. Polygenic risk scores were calculated using *P* value parameter 0.3 and recalibrated to have a mean of 0 and a unit increase corresponding to a doubling of risk for the disorder. The x-axis shows the mean polygenic risk score per decile. Affected patients are excluded.



Supplementary Figure 8: Bipolar disorder polygenic risk score decile versus variance in residual number of children in the total sample, males and females
Residual fecundity is number of children adjusted for year of birth, birth county of the last child, 5 principal components and sibship as a random effect. Polygenic risk scores were calculated using *P* value parameter 0.3 and recalibrated to have a mean of 0 and a unit increase corresponding to a doubling of risk for the disorder. The x-axis shows the mean polygenic risk score per decile. Affected patients are excluded.



Supplementary Figure 9: Major depression polygenic risk score decile versus variance in residual number of children in the total sample, males and females
Residual fecundity is number of children adjusted for year of birth, birth county of the last child, 5 principal components and sibship as a random effect. Polygenic risk scores were calculated using *P* value parameter 0.3 and recalibrated to have a mean of 0 and a unit increase corresponding to a doubling of risk for the disorder. The x-axis shows the mean polygenic risk score per decile. Affected patients are excluded.



Supplementary Figure 10: Schizophrenia polygenic risk score decile versus variance in residual number of children in the total sample, males and females
Residual fecundity is number of children adjusted for year of birth, birth county of the last child, 5 principal components and sibship as a random effect. Polygenic risk scores were calculated using *P* value parameter 0.3 and recalibrated to have a mean of 0 and a unit increase corresponding to a doubling of risk for the disorder. The x-axis shows the mean polygenic risk score per decile. Affected patients are excluded.

5. Genome-wide association study of suicide attempt in major psychiatric disorders

Authors

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Key words

suicide attempt, genome-wide association, genetics, polygenic

Abstract

Background: Suicide is a worldwide public health problem and the second leading cause of death among young adults. Over 90% of suicide attempters or victims have a psychiatric diagnosis, however twin and family studies suggest that the genetic aetiology of suicide attempt is partially distinct from that of the psychiatric disorders themselves. Genome-wide association studies (GWAS) to date have failed to identify replicable genetic associations with suicide attempt, although these studies have indicated that suicide attempt has a polygenic architecture. Here, we present the largest GWAS on suicide attempt to date, using cohorts of patients with major depressive disorder (MDD), bipolar disorder (BIP) and schizophrenia (SCZ), recruited from the Psychiatric Genomics Consortium.

Methods: Samples comprise 1622 suicide attempters and 8786 non-attempters with MDD, 3264 attempters and 5500 non-attempters with BIP and 1683 attempters and 2946 non-attempters with SCZ. GWAS on suicide attempt were performed by comparing suicide attempters versus non-attempters in each psychiatric disorder and then conducting meta-analysis between them. To further explore the genetic architecture of suicide attempt, polygenic risk scoring was used to test for pleiotropy between suicide attempt in these psychiatric disorders and the SNP heritability of suicide attempt in each disorder was calculated.

Results: Three genome-wide significant loci for suicide attempt were found: one in the GWAS of suicide attempt in MDD (rs45593736, $P = 2.61 \times 10^{-8}$, OR A allele = 2.38), one in the GWAS of suicide attempt in BIP, which strengthened in the meta-analysis of suicide attempt in mood disorders (top SNP rs28591567, $P = 3.11 \times 10^{-8}$, OR G allele 1.19) and an additional locus in the meta-analysis of suicide attempt in mood disorders (rs138689899, $P = 2.50 \times 10^{-8}$, OR T allele = 1.75). There were no significant associations with suicide attempt in SCZ or in the meta-analysis across all three disorders. Polygenic risk scoring provided no evidence of pleiotropy between suicide attempt in these psychiatric disorders and SNP heritability estimates for suicide attempt were non-significant.

Discussion: This study makes substantial progress in recruiting the large sample sizes required for robust genetic studies on suicide attempt. Replication of these genome-wide significant loci in independent cohorts is essential and is part of ongoing work. Non-significant polygenic risk scoring and SNP heritability results may reflect lack of power and increasing sample sizes further is desirable for future genetic studies on suicide attempt.

Introduction

Suicide is a worldwide public health problem with over 800,000 deaths due to suicide each year (World Health Organization, 2014). It is the second leading cause of death among young adults and rates of suicide are far exceeded by suicide attempts, which occur up to 20 times more frequently (World Health Organization, 2014). This represents a huge personal, social and economic burden, with the Centers for Disease Control and Prevention reporting that suicide costs the US economy $51 billion per year in healthcare and work-loss related costs (Centers for Disease Control and Prevention, 2015). These stark figures highlight the urgent need for improved prevention and treatment, however progress has been hampered by the lack of reliable methods for predicting suicidality and a poor understanding of its biological aetiology.

Over 90% of suicide attempters or victims have a psychiatric disorder, particularly mood disorders, schizophrenia and substance use disorders (Qin, 2011, Beautrais et al., 1996). The heritability estimate of suicidal behaviour from twin studies is 30-55% and twin and family studies suggest that the genetic aetiology of suicide attempt is partially distinct from that of the psychiatric disorders themselves (Voracek and Loibl, 2007, Brent and Mann, 2005). Several genome-wide association studies (GWAS) have been conducted on suicide attempt, by comparing attempters versus non-attempters with depression or bipolar disorder, to test for genetic variants contributing independently to suicide attempt (Willour et al., 2012, Schosser et al., 2011, Perlis et al., 2010, Mullins et al., 2014). These studies have failed to identify any replicable genetic associations, likely due to limited sample sizes which were underpowered to detect the small genetic effects typical for a single SNP. Other GWAS have focused on subjects recruited specifically on the basis of suicide attempt, rather than a particular psychiatric disorder, but again have not found any genetic associations (Galfalvy et al., 2015).

However, studies have indicated that suicide attempt has a polygenic architecture, as polygenic risk scores for suicide attempt generated from the results of a previous GWAS showed modest association with suicide attempt in an independent sample, consistent with the presence of small genetic effects that the original GWAS was underpowered to detect at genome-wide significance (Mullins et al., 2014). In the current study, we present the largest GWAS on suicide attempt to date, comparing a total of 6,569 suicide attempters and 17,232 non-attempters, with major depressive disorder (MDD), bipolar disorder (BIP) or schizophrenia (SCZ), recruited from the Psychiatric Genomics Consortium.

Methods

Subjects and phenotype definition

Subjects were drawn from 16 MDD cohorts, 21 BIP cohorts and 9 SCZ cohorts which are part of the Psychiatric Genomics Consortium (PGC) and had information available on suicide attempts. Only cases affected with psychiatric disorders were included in this study and all cases were defined using structured psychiatric interviews according to international consensus criteria (DSM-IV, ICD-9, or ICD-10) (American Psychiatric Association, 1994, World Health Organization, 1978, World Health Organization, 1992). Supplementary Tables 1-3 summarise the source, inclusion and exclusion criteria for cases in each cohort. All subjects were of European ancestry. Suicide attempt (SA) was defined in each cohort using items from structured clinical interviews (Supplementary Table 4). Lifetime suicide attempt was defined across cohorts as a deliberate act of self-harm with at least some intent to result in death. Individuals missing information on suicide attempt were excluded. Across the MDD, BIP and SCZ datasets, there were a total of 6,569 suicide attempters and 17,232 non-attempters (Table 1).

Genotyping, quality control and imputation

Cohorts were genotyped following their local protocols, after which standardised quality control and imputation were performed centrally using the PGC 'Ricopili' pipeline (https://sites.google.com/a/broadinstitute.org/ricopili/), for each cohort separately. These procedures have been described in detail previously (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). Briefly, the quality control parameters for retaining SNPs and subjects were: SNP missingness < 0.05 (before sample removal), subject missingness < 0.02, autosomal heterozygosity deviation (F_{het} < 0.2), SNP missingness < 0.02 (after sample removal), difference in SNP missingness between psychiatric cases and healthy controls < 0.02, SNP Hardy-Weinberg equilibrium ($P > 10^{-10}$ in psychiatric cases) and minor allele frequency ≥ 0.01. Genotype imputation was performed using the pre-phasing/ imputation stepwise approach implemented in IMPUTE2/ SHAPEIT (chunk size of 3 Mb and default parameters) to the 1000 Genomes Project reference panel (Howie et al., 2011, Delaneau et al., 2012, 1000 Genomes Project Consortium, 2010). SNPs with an imputation INFO-score < 0.6 were excluded. The numbers of SNPs analysed were 8482392, 8807006 and 8814543 in the MDD, BIP, and SCZ datasets respectively.

Statistical analysis

GWAS on suicide attempt were performed using PLINK2 by comparing imputed marker dosages under an additive logistic regression model between suicide attempters and non-attempters in

each cohort separately (Chang et al., 2015). Five principal components, generated using EIGENSTRAT were used as covariates in all GWAS to control for population stratification (Price et al., 2006). There was no evidence of stratification artifacts or uncontrolled test statistic inflation in the results from any cohort (e.g. λ_{GC} was 0.87 - 1.01). Within each psychiatric disorder, meta-analysis of the results from all cohorts was performed using an inverse-weighted fixed effects model in METAL, to obtain GWAS results for suicide attempt in MDD, suicide attempt in BIP and suicide attempt in SCZ (Willer et al., 2010). A fixed effects meta-analysis was also conducted between the GWAS results of suicide attempt in the three psychiatric disorders, the mood disorders only and between the results in BIP and SCZ. Assuming a discrete trait case-control model, the Genetic Power Calculator was used to determine the power to detect associations at genome-wide significance ($P < 5 \times 10^{-8}$), for the meta-analysis of 6,569 suicide attempters and 17,232 non-attempters, across the three psychiatric disorders (Purcell et al., 2003). The GWAS has 78% power to detect an allele with frequency 0.2 and effect size 1.1 at genome-wide significance.

Polygenic risk scoring was used to test for genetic overlap between suicide attempt in these psychiatric disorders. The results of three GWAS (SA in MDD, SA in BIP and SA in SCZ) were used in turn as discovery studies and the remaining two disorders were used as separate test datasets, resulting in a total of 6 polygenic risk scoring analyses. PRSice software was used to generate the PRS, according to standard protocol (Euesden et al., 2015). The GWAS results from each discovery study were pruned for linkage disequilibrium (LD) using the P value informed clumping method in PLINK (--clump-p1 1 --clump-p2 1 --clump-r2 0.1 --clump-kb 250). This preferentially retains SNPs with the strongest evidence of association and removes SNPs in LD ($r^2 > 0.1$) that show weaker evidence of association within 250Kb windows. Subsets of SNPs were selected from the results at nine increasingly liberal P value thresholds ($P < 0.0001$, $P < 0.001$, $P < 0.01$, $P < 0.05$, $P < 0.1$, $P < 0.2$, $P < 0.3$, $P < 0.4$, $P < 0.5$). Sets of alleles, weighted by their log odds ratios (OR) from the discovery GWAS, were summed into a PRS for each individual in the test datasets using PLINK. The PRS for suicide attempt was tested for ability to predict suicide attempter versus non-attempter status in the other two psychiatric disorders separately using logistic regression. The regression model also included five principal components and a covariate for each cohort in the test dataset. The amount of variance explained by the PRS was calculated as Nagelkerke's pseudo-R^2. In total, six PRS analyses were conducted, hence the Bonferroni corrected significance threshold is 0.008. Statistical power for polygenic risk scoring was calculated using AVENGEME software (Dudbridge, 2013, Palla and Dudbridge, 2015).

The variance in suicide attempt explained by genotyped SNPs (SNP heritability, h^2_{SNP}) was assessed using genomic-relatedness-based restricted maximum-likelihood (GREML), implemented in GCTA software (Yang et al., 2011). The SNP probabilities were converted to best guess data with a genotype call probability cut-off of 0.8. HapMap 3 SNPs with an INFO score ≥ 0.6 were used to calculate the genetic relatedness matrix (GRM) using PLINK2, including individuals with relatedness < 0.05 (Chang et al., 2015). Ancestry informative principal components were calculated using GCTA (Yang et al., 2011). The GRM was based on a total of 1166347 SNPs in the MDD dataset, 1172705 SNPs in the BIP dataset and 1143070 SNPs in the SCZ dataset. Covariates included 20 principal components calculated using GCTA (because GRM-based analyses are more sensitive to population stratification than PRS analyses) and a covariate for each cohort within a disorder. The h^2_{SNP} of SA in each psychiatric disorder was calculated using GCTA and power calculations were performed using the GCTA-GREML power calculator (Visscher et al., 2014).

Results

Sample characteristics

Table 1 shows the number and proportion of suicide attempters and non-attempters within each psychiatric disorder. The numbers in individual cohorts are shown in Supplementary Tables 5-7.

Table 1: Sample characteristics

Disorder	*N* cohorts	*N* Suicide attempters (%)	*N* Non-attempters (%)	*N* Female Suicide attempters (%)	*N* Female Non-attempters (%)
Major depressive disorder	16	1622 (16%)	8786 (84%)	1155 (71%)	5808 (66%)
Bipolar disorder	21	3264 (37%)	5500 (63%)	2097 (66%)	2971 (56%)
Schizophrenia	9	1683 (36%)	2946 (64%)	660 (39%)	924 (31%)
Total		6569 (28%)	17232 (72%)	3912 (60%)	9703 (56%)

Genome-wide association studies

A GWAS of suicide attempters versus non-attempters was performed in the MDD, BIP and SCZ datasets separately. In the GWAS of suicide attempt in MDD, one SNP reached genome-wide significance: rs45593736, P = 2.61 x 10^{-8}, OR A allele = 2.38 (Table 2). This SNP is in an intron of the *ARL5B* (ADP-Ribosylation Factor-Like 5B) gene. An insertion-deletion polymorphism on chromosome 4 met genome-wide significance in the GWAS of suicide attempt in BIP, with the

direction of effect highly consistent across the 21 BIP cohorts: chr4_23273116_D, P = 1.15×10^{-8}, OR for the deletion = 1.29 (Table 2). This is an intronic variant in the non-coding RNA *LOC105374524*. In the GWAS of suicide attempt in SCZ, there were no SNPs reaching genome-wide significance.

Meta-analysis of the GWAS results for suicide attempt across all three disorders, produced no genome-wide significant results (Supplementary Table 11). Figure 1 shows the Manhattan plot from the meta-analysis of suicide attempt in mood disorders. In this analysis, there were 10 genome-wide significant SNPs from two independent loci (Table 2). The top association was rs138689899 on chromosome 2, P = 2.50×10^{-8}, OR T allele = 1.75. This is an intergenic SNP between the *IWS1* and *MYO7B* genes. The other significant locus was on chromosome 4 and is also in *LOC105374524.* It is in high LD (R^2= 0.83) with the insertion-deletion polymorphism identified in the GWAS of SA in BIP. The most significant SNP was rs28591567, P = 3.11×10^{-8}, OR G allele 1.19 (Table 2). Figure 2 is a regional association plot of *LOC105374524* showing P values from the meta-analysis of suicide attempt in mood disorders. This locus was not associated with suicide attempt in schizophrenia or MDD, although in MDD the direction of effect was consistent (rs28591567, P = 0.03, OR allele G allele = 1.11) (Supplementary Table 14). A meta-analysis was also performed between SA in BIP and SCZ to investigate psychotic suicide attempts, but there were no significant associations (Supplementary Table 13). Manhattan plots and tables of the top results from all GWAS are in the Supplementary Materials.

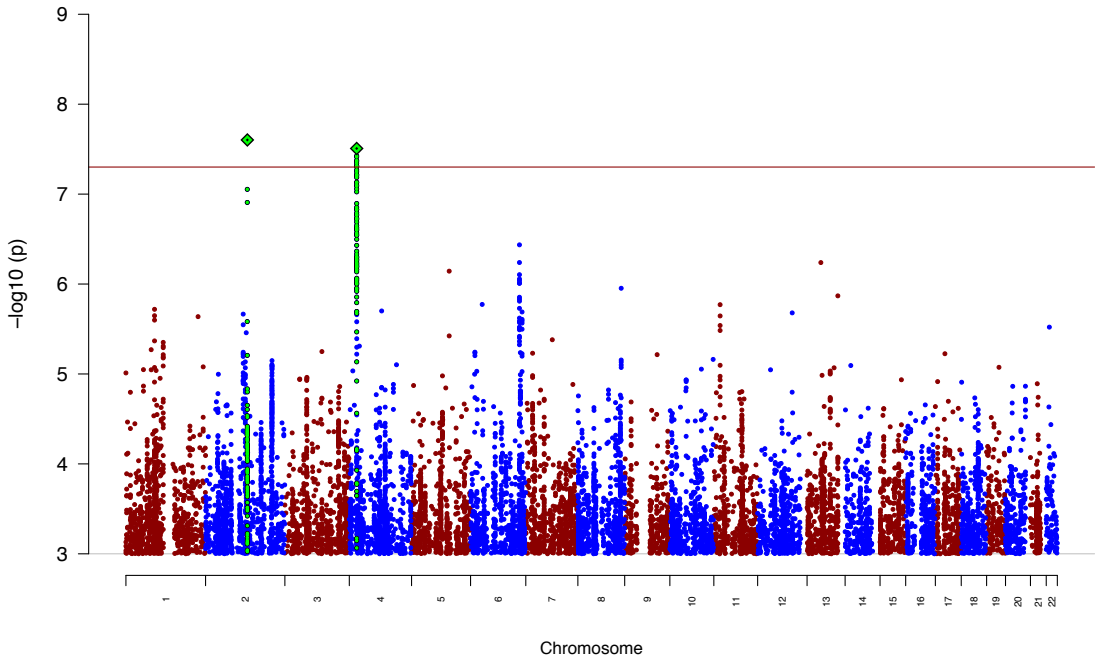

Figure 1: Manhattan plot of suicide attempt in mood disorders.

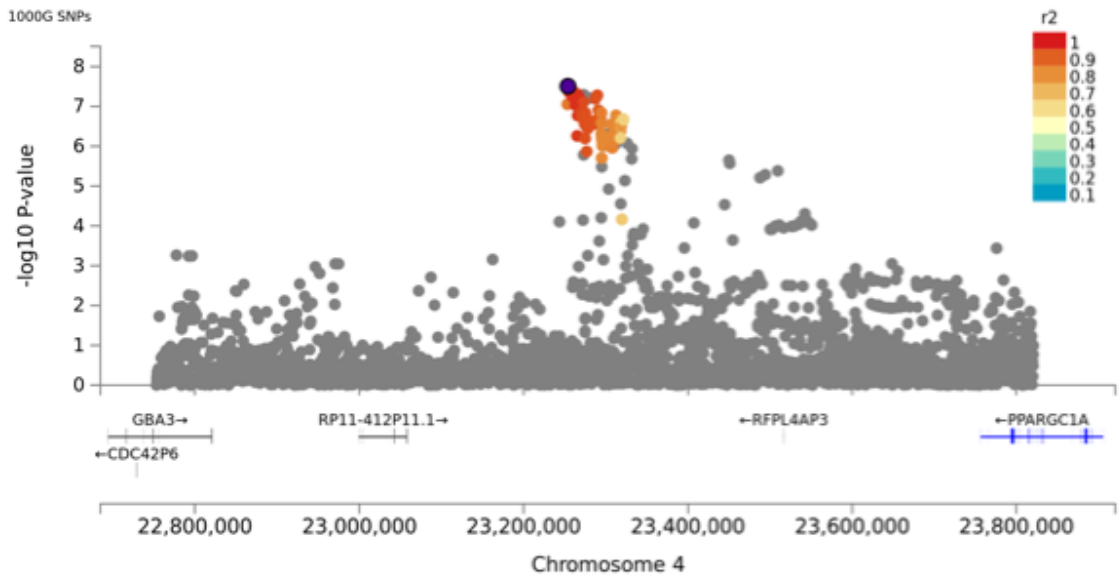

Figure 2: Regional association plot of *LOC105374524* showing P values from meta-analysis of suicide attempt in mood disorders. Top lead SNP = rs28591567.

Table 2: Genome-wide significant loci for suicide attempt showing the most significant variant from each genomic region

Dataset	Variant	CHR	BP	Tested allele	Allele freq	INFO score	P value	OR (C.I.)	Direction in each cohort	Genes (distance from SNP in Kb)
MDD	rs45593736	10	18954937	A	0.02	0.89	2.61E-08	2.38 (1.75-3.23)	?++++-++?+-?++++	*ARL5B* (intronic)
BIP	chr4_23273116_D*	4	23273116	D	0.20	0.91	1.15E-08	1.29 (1.18-1.41)	+++++++++++++--+++++	*LOC105374524*
Mood disorders	rs138689899	2	128288162	T	0.02	0.91	2.50E-08	1.75 (1.44-2.14)	++	*IWS1*(3.7), *MYO7B* (107.1)
Mood disorders	rs28591567*	4	23253912	G	0.22	0.95	3.11E-08	1.19 (1.12-1.27)	++	*LOC105374524*

CHR, chromosome; BP, basepair position; freq, frequency; OR, odds ratio; CI, confidence interval; MDD, major depressive disorder;

BIP, bipolar disorder. *chr4_23273116_D and rs28591567 LD R2 = 0.83

Polygenic risk scoring and SNP heritability

Polygenic risk scoring was performed to investigate the genetic relationship between suicide attempt in MDD, BIP and SCZ. Polygenic risk scores for SA in one psychiatric disorder were not significantly associated with suicide attempt in another disorder (Supplementary Figure 6). The maximum variance explained was 0.09% using the PRS for SA in BIP to predict suicide attempt in schizophrenia (P = 0.07). GREML was used to calculate the h^2_{SNP} of suicide attempt in each psychiatric disorder. The h^2_{SNP} estimate of suicide attempt in MDD was 0.03 (SE = 0.03, P = 0.19), in BIP was 0.02 (SE = 0.03, P = 0.25) and in SCZ was 0.10 (SE = 0.07, P = 0.06). The power to detect

these h^2_{SNP} values for suicide attempt was 6%, 6% and 14% in the MDD, BIP and SCZ datasets respectively. Statistical power for polygenic risk scoring given the observed h^2_{SNP} estimates ranged from 5-22%.

Discussion

This GWAS on suicide attempt is the largest to date, combining samples of suicide attempters and non-attempters across three major psychiatric disorders, recruited from the Psychiatric Genomics Consortium. Three independent loci were associated with suicide attempt. The strongest support was for the chromosome 4 locus in *LOC105374524*. Here, one variant reached genome-wide significance in the GWAS of SA in BIP and support for the region strengthened in the meta-analysis of SA in mood disorders, with 9 SNPs reaching genome-wide significance and 89 others in high LD with $P < 5 \times 10^{-6}$ (Figure 2). This region was not implicated in the GWAS of suicide attempt in schizophrenia and it has not been associated with MDD, BIP or SCZ in the latest GWAS conducted by the PGC (Supplementary Table 14). This evidence together suggests that the association is specific to suicide attempt in mood disorders. *LOC105374524* is a non-coding RNA, located approximately 500Kb downstream of the *GBA3* (Glucosylceramidase Beta 3) gene and upstream of *PPARGC1A*, which is a transcriptional coactivator that regulates the genes involved in energy metabolism.

Replication of the genome-wide significant loci in independent samples is essential and is part of planned future work. Through collaboration, we will investigate these associations in a large sample of suicide attempters with mood disorders drawn from the Danish population registry. The UK Biobank Study, which is a large population-based genetic study of disease, will also provide data from a mental health questionnaire (Sudlow et al., 2015). This will include a wealth of information on suicide attempt, suicidal ideation and non-suicidal self-injury, which will provide future opportunities for replication and to investigate the genetic relationships between these phenotypes and the psychiatric disorders.

The meta-analysis of suicide attempt across all three psychiatric disorders found no genetic associations and polygenic risk scoring showed no evidence of pleiotropy between suicide attempt in different disorders, explaining a negligible amount of variance. Lack of power in the discovery GWAS is one probable explanation for this, and indeed power calculations indicated that these analyses were underpowered. However, there may also be genetic heterogeneity in suicide attempt, for example between depressive versus psychotic suicide attempts. This has

implications for future genetic studies. One strategy could be to focus on more homogenous samples, while an alternate approach is to recruit even larger sample sizes through consortia, which could include existing cohorts with other psychiatric disorders where suicide attempt is prevalent, such as substance use disorders or eating disorders. As seen in GWAS on MDD, both of these approaches have their merits, as large samples sizes can increase statistical power despite heterogeneity (Mullins and Lewis, 2017).

The within-case comparison of attempters versus non-attempters used for these GWAS was utilised to detect associations specific for suicide attempt and was informed by twin and family studies which consistently indicate a genetic component to suicide attempt which is independent of the psychiatric disorders themselves (Voracek and Loibl, 2007, Brent and Mann, 2005). Several twin studies have calculated that suicide attempt is moderately heritable, although one study showed that after adjusting for other psychiatric disorders, heritability decreased to 17%, which would reduce statistical power to detect genetic associations (Voracek and Loibl, 2007, Fu et al., 2002). The epidemiological evidence suggests that suicidality may be better considered as a comorbidity rather than an intrinsic symptom of psychiatric disorders. The Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-5) lists Suicidal Behavior Disorder as a condition for further study and this nomenclature and clear criteria should lead to improved identification and documentation of this serious condition, which will aid patient care and future research (American Psychiatric Association, 2013). The subjects included in this study were not ascertained primarily for suicide attempt and therefore detailed information such as the number of suicide attempts, method, medical consequences or medication is not available for all cohorts. We focused on lifetime suicide attempt in order to maximise sample size and increase statistical power.

This is the first consortium-based GWAS on suicide attempt and makes progress in recruiting the large sample sizes required for robust genetic studies on this phenotype. However, further increases in sample size are essential to fully interrogate the common genetic architecture of suicide attempt and this will necessitate increased collaboration. There is an urgent need to better understand the aetiology of suicide attempt and this GWAS makes initial progress in identifying genetic variants which may be involved. The number of genetic associations is expected to increase with sample size and even genetic associations with small effect sizes have the potential to provide invaluable biological insights, leading to much-needed treatments and preventions for suicidality.

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Supplementary material
Genome-wide association study of suicide attempt in major psychiatric disorders

Table S1: Description of 16 major depressive disorder cohorts

Cohort (References)	PGC label	Country	Ascertainment and Evaluation of Cases	Inclusion criteria (lifetime) of Cases	Exclusion criteria (lifetime) of Cases
BOMA 1-3	boma	Germany	Consecutive inpatients; SCID or SADS interview; medical records	DSM-IV MDD; German ancestry; age ≥ 18	BIP, hypomania, NAP, MDD related to SUD
CoFaMS 4	cof3	Australia	Opportunistic; inpatient & outpatient; SCID or MINI	DSM-IV MDD	BIP, NAP, MDD related to SUD
PsyCoLaus 5	col3	Switzerland	Random population sample; DIGS	DSM-IV MDD, age 35-66	BIP, hypomania, NAP, MDD related to SUD
GenRED1 6,7	gens	USA	Opportunistic; DIGS3; medical records or informant (subset)	DSM-IV MDD (recurrent or episode >3 yrs) & onset <31 yrs; FHx MDD in sibling or parent	BIP, NAP, mod-severe ID; FHx BIP; if SUD, MDD onsets without <2y of sobriety
GenRED2 6	grnd	USA	Opportunistic; DIGS; medical records or informant (subset)	DSM-IV MDD (recurrent or episode >3 yrs) & onset <31 yrs; FHx MDD in sibling or parent	BIP, NAP, mod-severe ID; FHx BIP; if SUD, MDD onsets without <2y of sobriety
GSK/MPIP 8	gsk2	Germany	Inpatients; SCAN	DSM-IV MDD (recurrent, mod-severe)	BIP, NAP, SUD, mood-incongruent psychosis, OCD, PTSD, secondary MD
MARS 9-11	mmi2 mmo4	Germany	Inpatients; CIDI	DSM-IV MDD	BIP, SUD, secondary MD, severe medical conditions
NESDA/NTR: NESDA 12,13	nes1	Netherlands	Psychiatric outpatients, primary care, & population; CIDI	DSM-IV MDD	BIP, NAP, SUD
NESDA/NTR: NTR 12,13	nes1	Netherlands	Twin registry; longitudinal MDD sx; CIDI (subset)	DSM-IV MDD	Mania (if interviewed)
QIMR 14,15	qi3c qi6c qio2	Australia	Australian Twin Registry (proband most severe, sx, or earlier onset); SSAGA	DSM-IV MDD	MDD related to SUD
RADIANT-UK 16	rad3	UK	UK outpatients from DeNT, DeCC, GENDEP studies; SCAN	DSM-IV MDD (recurrent in DeCC & DeNT; MDD FHx in DeNT)	BIP, NAP, MDD related to SUD; BIP FHx
RADIANT-GER 16	rage	Germany	German outpatients from DeNT, DeCC, GENDEP studies; SCAN	DSM-IV MDD (recurrent in DeCC & DeNT; MDD FHx in DeNT)	BIP, NAP, MDD related to SUD; BIP FHx
SHIP 0 17	shp0	Germany	Study of Health in Pomerania; CIDI	DSM-IV MDD	BIP, MDD related to SUD
STAR*D 18	stm2	USA	Outpatients in clinical trial; clinical interviews	DSM-IV MDD	BIP, NAP

Abbreviations: SCID=Structured Clinical Interview for DSM-IV, SADS=Schedule for Affective Disorders and Schizophrenia, MDD = major depressive disorder, BIP = bipolar disorder, NAP=non-affective psychosis, SUD = substance use disorder, MINI = MINI International Neuropsychiatric Interview, DIGS=Diagnostic Interview for Genetic Studies, Fhx = family history, ID = intellectual disability, SCAN = Schedules for Clinical Assessment in Neuropsychiatry, OCD = obsessive compulsive disorder, PTSD = post-traumatic stress disorder, CIDI = Composite International Diagnostic Interview, sx = symptoms

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Table S2: Description of 21 bipolar disorder cohorts

Cohort (References)	PGC label	Country	Ascertainment and Evaluation of Cases	Inclusion criteria (lifetime) of Cases	Exclusion criteria (lifetime) of Cases
Bonn/ Mannheim 1-4	bonn	Germany	Consecutive admissions to in-patent units, SCID-I, SADS-L, medical records, FHx, OPCRIT	DSM-IV BIP I or BIP II	
Trinity College Dublin 5	dub1	Ireland	Hospitals and Community psychiatric facilities, SCID, case note review	DSM-IV BIP I	
FaST, TGEN1, TGEN2 6	fat2	USA	Hospitals, ADE, MINI	DSM-IV BIP I or BIP II	
French PGC2 6	fran	France	DIGS, FIGS, medical case notes, mood scales, self-rating questionnaires assessing dimensions	DSM-IV BIP I or BIP II	
GSK 7	gsk1	UK, Canada	Advertisements in hospitals, clinics, primary care physician offices, patient support groups, SCAN CATEGO algorithm	DSM-IV or ICD-10 BIP I or BIP II	Dx of intravenous drug dependency/use, mood incongruent psychotic sx, manic episodes only with alcohol/substance abuse/dependence/medical illnesses/medications
Mayo Clinic 8	may1	USA	Mayo Clinic Bipolar Biobank, patients ascertained through routine clinical appointments, in-patients in mood disorder units and recruitment advertising, SCID	DSM-IV-TR BIP I/ BIP II/ schizoaffective	
Pritzker Neuropsychiatric Disorders Research Consortium 7, 9	mich	USA	NIMH Genetics Initiative Repository, DIGS, FIGS, medical record review	DSM-III or IV BIP I	Suspected major depression
STEP1 5,7	stp1	USA	ADE, MINI	DSM-IV BIP I	
STEP2	st2c	USA	Hospitals, ADE, MINI	DSM-IV BIP I or BIP II	
TOP7 10	top7	Norway	Out-patient and in-patient psychiatric units, SCID-I, case note review, follow up interview	DSM-IV BIP I, BIP II, SAB, BIP-NOS	IQ score < 70
TOP8 10	top8	Norway	Out-patient and in-patient psychiatric units, SCID-I, case note review, follow up interview	DSM-IV BIP I, BIP II, SAB, BIP-NOS	IQ score < 70
UCL 5, 11	uclo	UK	Clinical diagnosis according to UK National Health Service (NHS) psychiatrists at interview, SADS-L, OPCRIT	DSM-IV BIP I	
UMEA	ume4	Sweden	MINI, DIGS, FIGS, SCAN	DSM-IV-TR BIP	
WTCCC 5, 7, 12	wtcc	UK	Individuals in contact with mental health services, SCAN	RDC BIP I, BIP II, SAB, BIP-NOS	
GAIN 7, 13	gain	USA	Multiplex families, sibling pair families or individuals, DIGS, FIGS, medical records	DSM IIR & IV BIP I or SAB	
BOMA-Moods 14	bmg2	Germany	Consecutive admissions to in-patient units, AMDP, medical records, family history, OPCRIT	DSM-IV lifetime BIP	
BOMA-Moods 14	bmg3	Germany	Recruited from psychiatric hospitals, AMDP, CID-S, SADS-L, SCID, medical records, family history, OPCRIT	DSM-IV lifetime BIP	
BOMA-Moods 14	bmpo	Poland	Recruited from Department of Psychiatry, SCID	DSM-IV lifetime BIP	
BOMA-Moods 14	bmsp	Spain	Recruited from hospital mental health departments, SADS-L, OPCRIT, medical records, FISC	DSM-IV and RDC BIP	
Nova Scotia	hal2	Canada	Recruited from specialty mood disorder clinics, SADS-L	DSM-IV and RDC BIP	
Romania 15	rom3	Romania	Consecutive admissions to psychiatric hospital, DIGS, FIGS, medical records, family reports	DSM-IV BIP I	

Abbreviations: SCID = Structured Clinical Interview for DSM-IV, SADS-L = Schedule for Affective Disorders and Schizophrenia Lifetime Version, FHx = family history, OPCRIT = Operational Criteria Checklist, BIP = bipolar disorder, ADE = Affective Disorders Evaluation, MINI = MINI International Neuropsychiatric Interview, DIGS = Diagnostic Interview for Genetic Studies, FIGS = Family Interview for Genetic Studies, SCAN = Schedules for Clinical Assessment in Neuropsychiatry, Dx = diagnosis, sx = symptoms, SAB = Seasonal affective disorder, BIP-NOS = bipolar disorder not otherwise specified, AMDP = Association Methodology and Documentation in Psychiatry, CID-S = Composite International Diagnostic Screener, FISC = Family Informant Schedule and Criteria, RDC = Research Diagnostic Criteria

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Table S3: Description of 9 schizophrenia cohorts

Cohort (References)	PGC label	Country	Ascertainment and Evaluation of Cases	Inclusion criteria (lifetime) of Cases	Exclusion criteria (lifetime) of Cases
Bonn/ Mannheim 1	boco	Germany	Consecutive hospital admissions, SCID, SADS-L, OPCRIT, medical records, FHx	DSM-IV SCZ	
Bulgaria 2	butr	Bulgaria	Family trios where proband had SCZ/ SA, SCAN	DSM-IV SCZ / SA	Mental retardation
Denmark 1	denm	Denmark	Psychiatric departments and twin pair studies, OPCRIT	ICD-10 SCZ	Mania/bipolar illness
Molecular Genetics of Schizophrenia 3	mgs2	USA, Australia	Clinical settings and community residences, DIGS 2.0, FIGS 2.0, Medical records	DSM-IV SCZ / SA	
Munich 1	munc	Germany	Cases diagnosed with SCZ from the Munich area, SCID interview	DSM-IV SCZ	Head injury/ neurological diseases
Portugal 4	port	Portugal	Probands from families segregating SCZ, DIGS, SIS, SANS, SAPS, OPCRIT	DSM-IV SCZ	Bipolar disorder
Thematic Organized Psychosis Research	top8	Norway	Out-patient and in-patient psychiatric units, SCID-I interview	DSM-IV SCZ/ SA/ schizophreniform disorder	IQ score < 70
UCLA 1	ucla	Netherlands	Inpatients and outpatients recruited through psychiatric hospitals and institutions, CASH	DSM-IV SCZ	Short-term drug-induced psychoses, psychoses with learning disability/ head injury, other symptomatic psychoses
University College London 4	uclo	UK	SCZ diagnosis recorded in medical case-history, SADS-L, RDC	ICD-10 SCZ	SA, bipolar disorder, schizomania

Abbreviations: SCID = Structured Clinical Interview for DSM-IV, SADS = Schedule for Affective Disorders and Schizophrenia, OPCRIT = Operational Criteria Checklist, FHx = family history, SCZ = schizophrenia, SA = schizoaffective disorder, SCAN = Schedules for Clinlical Assessment in Neuropsychiatry, DIGS = Diagnostic Interview for Genetic Studies, FIGS = Family Interview for Genetic Studies, SIS = Kendler's Structured Interview for Schizotypy, SANS = Schedule for the Assessment of Negative Symptoms, SAPS = Schedule for the Assessment of Positive Symptoms, CASH = Comprehensive Assessment of Symptoms and History, RDC = Research Diagnostic Criteria

References	*Number*	*Citation*
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Table S4: Items on suicide from psychiatric interviews

Psychiatric Interview	Section/ Question	Information Collected
SCAN (Schedules for Clinical Assessment in Neuropsychiatry)	6.011 Suicide attempt and self-harm during episode of depression	0=absent, 1=deliberately considered suicide or self-injury but made no attempt, 2= injured self or made an attempt but no serious harm results, 3 = as 2 but with serious self-harm, 4 = made an attempt at suicide designed to result in death
SCID (Structured Clinical Interview for DSM-IV)	Sections on Depression, Mania, Mixed states	Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide
SADS (Schedule for Affective Disorders and Schizophrenia)	Section O Suicidal Behavior	Ever made a suicide attempt, describe the most serious attempt, most serious attempt is rated by the interviewer in terms of intent and lethality
DIGS (Diagnostic Interview for Genetic Studies)	Section O Suicidal Behavior	Ever made a suicide attempt, describe the most serious attempt, medical treatment or hospitalisation required, whether the patient wanted to die or thought they would die, most serious attempt is rated by the interviewer in terms of intent and lethality
OPCRIT (Operational Criteria Checklist)	Past psychiatric history	Ever made suicide attempt
MINI (MINI International Neuropsychiatric Interview)	Section C Suicidality	Lifetime suicide attempt, in the past month thoughts about suicide, suicide plan, suicide attempt, hoped to survive or expected to die
CIDI (Composite International Diagnostic Interview)	Section on Major Depression	During worst two weeks in the last 12 months - thought about commiting suicide, suicide plan, suicide attempt
SSAGA (Semi-Structured Assessment for the Genetics of Alcoholism)	Section I Depression, Section N Suicidal Behavior	Thoughts of death or suicide, sucide plan, suicide attempt, describe the most serious attempt, method, medical treatment, hospitalisation, whether the patient wanted to die or thought they would die, interviewer rates both the lethality and intent from unclear to extreme
FIGS (Family Interview for Genetic Studies)	During depression	Did the family member talk about death or suicide, try suicide
CASH (Comprehensive Assessment of Symptoms and History)	Major Depressive Syndrome	Thoughts about death and suicide, plus possible wishes to be dead, suicide plans, suicide attempts, rated from mild to severe

Table S5: Summary of suicide attempt in major depressive disorder cohorts

Cohort	*N* Suicide attempters	*N* Non-attempters
CoFaMS	27	74
PsyCoLaus	65	442
GenRED2	168	653
GSK MPIP	115	763
MARS 650	137	407
MARS OMNIex	38	187
NTR/NESDA	229	1146
QIMR I317	61	521
QIMR I610	32	263
QIMR COEX	48	299
RADIANT-UK	150	1424
RADIANT-Ger	35	276
STAR*D	126	807
BOMA	170	361
SHIP 0	18	348
GenRED1	203	815
Total	**1622**	**8786**

Table S6: Summary of suicide attempt in bipolar disorder cohorts

Cohort	*N* Suicide attempters	*N* Non-attempters
Bonn/ Mannheim	241	365
Trinity College Dublin	26	26
FaST, TGEN1, TGEN2	120	124
French PGC2	185	254
GSK	77	505
Mayo Clinic	307	610
Pritzker Neuropsychiatric Disorders Research Consortium	161	310
STEP2	170	363
STEP1	392	453
TOP7	116	207
TOP8	48	94
UCL	182	74
UMEA	74	124
WTCCC	423	673
GAIN	233	277
BOMA-Moods Germany	62	119
BOMA-Moods Germany	132	234
Boma-Moods Poland	150	251
Boma-Moods Australia	22	66
Nova Scotia Canada	78	223
Romania	65	148
Total	**3264**	**5500**

Table S7: Summary of suicide attempt in schizophrenia cohorts

Cohorts	*N* Suicide attempters	*N* Non-attempters
Bonn/ Mannheim	287	310
Bulgaria	103	208
Denmark	28	98
Molecular Genetics of Schizophrenia	754	1202
Munich	156	263
Portugal	80	243
Thematic Organized Psychosis Research	100	214
UCLA	86	304
University College London	89	104
Total	**1683**	**2946**

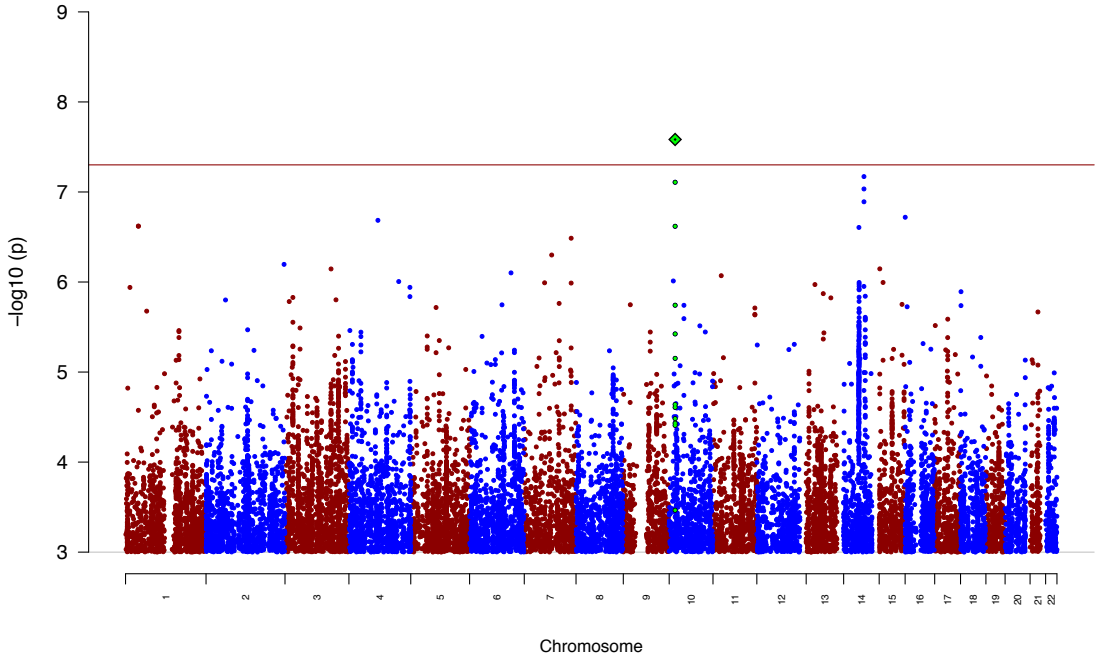

Figure S1: Manhattan plot of suicide attempt in major depressive disorder

Table S8: Top 20 results from GWAS of suicide attempt in major depressive disorder showing the most significant SNP from each genomic region

Variant	CHR	BP	A1/A2	A1 freq attempters	A1 freq non-attempters	P value	OR (C.I.)	Direction in each cohort
rs45593736	10	18954937	A/G	0.02	0.01	**2.61E-08**	2.38(1.75-3.23)	+++?++++-++?+-?+
rs111625585	14	82804332	T/C	0.08	0.06	6.75E-08	1.57(1.33-1.84)	+++--+++++-+++-+
rs116428372	16	589359	A/G	0.07	0.06	1.91E-07	1.74(1.41-2.14)	++++++++-+++-+++
rs77033326	4	89777618	A/G	0.03	0.02	2.07E-07	2.24(1.65-3.03)	+-++-+-+-++-++++
rs183414028	1	40442026	T/C	0.98	0.99	2.39E-07	0.39(0.27-0.56)	----------+-+---
rs113330417	14	67249421	A/G	0.96	0.98	2.48E-07	0.57(0.46-0.71)	----------+---
rs111367251	7	144968289	C/G	0.98	0.99	3.26E-07	0.45(0.33-0.61)	-?-+------+----+
rs62460873	7	84977966	T/C	0.98	0.99	5.01E-07	0.37(0.25-0.54)	?--???-----++---
rs186736781	2	240473090	T/C	0.03	0.02	6.37E-07	2.27(1.64-3.13)	+?+?-+-++++-+---
chr15_24344805_D	15	24344805	D/I3	0.37	0.41	7.14E-07	0.79(0.73-0.87)	----------+-+-+-
rs111326206	3	142438431	T/C	0.95	0.97	7.16E-07	0.62(0.51-0.75)	-+-----+--+?--+-
chr6_128178230_I	6	128178230	I2/D	0.08	0.06	7.92E-07	1.58(1.32-1.90)	--------+-------
rs184924771	11	25885205	A/C	0.98	0.99	8.51E-07	0.39(0.27-0.57)	-?-?+--?+-??-?--
rs113386487	10	13358583	A/T	0.97	0.98	9.73E-07	0.47(0.35-0.64)	-?-??---?-?+?--+
rs13137453	4	153907879	A/G	0.97	0.98	9.88E-07	0.46(0.34-0.63)	-?-?+---?---+---
rs9972552	15	34396913	A/C	0.03	0.02	1.01E-06	2.18(1.59-2.98)	+-+++-++++-+++++
rs191852465	7	63164142	T/C	0.96	0.97	1.02E-06	0.46(0.34-0.63)	-++----------+-
chr13_46256859_D	13	46256859	D/I6	0.02	0.01	1.07E-06	2.27(1.63-3.16)	--++?+?-++-+++++
rs145440507	4	188400537	A/T	0.98	0.99	1.15E-06	0.37(0.25-0.55)	-?-???-??--?-??-
rs76347430	1	14395819	A/G	0.97	0.98	1.15E-06	0.50(0.38-0.66)	?-----+-+--++--+

CHR, chromosome; BP, basepair position; freq, frequency; OR, odds ratio; CI, confidence interval

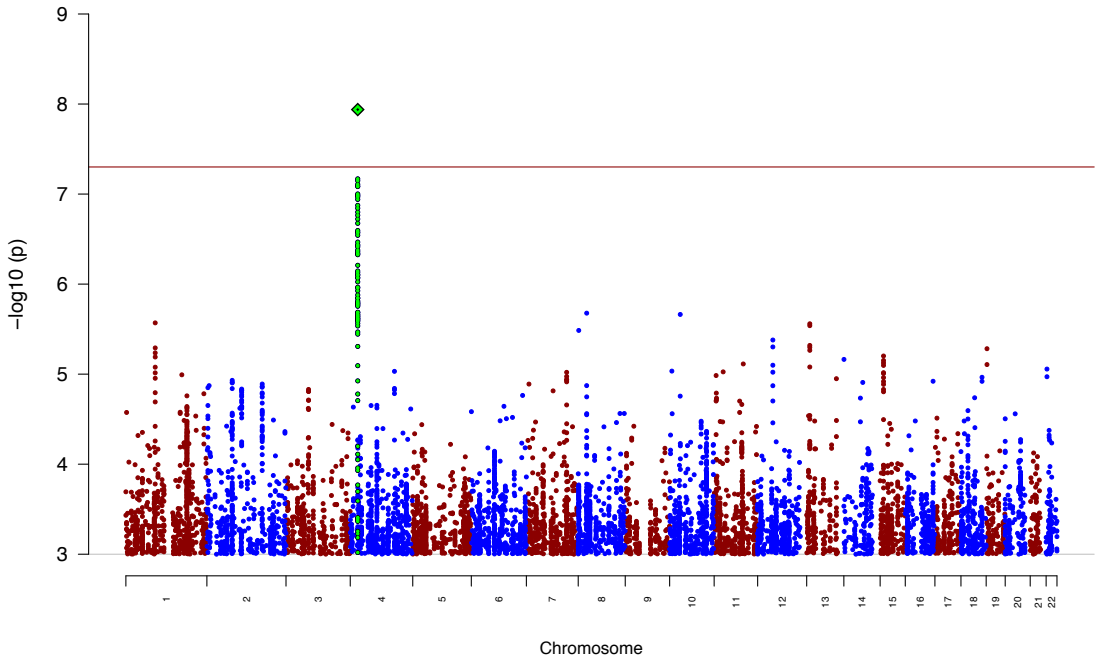

Figure S2: Manhattan plot of suicide attempt in bipolar disorder

Table S9: Top 20 results from GWAS of suicide attempt in bipolar disorder showing the most significant SNP from each genomic region

Variant	CHR	BP	A1/A2	A1 freq attempters	A1 freq non-attempters	P value	OR (C.I.)	Direction in each cohort
chr4_23273116_D	4	23273116	D/I10	0.20	0.17	1.15E-08	1.29(1.18-1.40)	++++++++++++++--+++++
rs1052873	8	27667793	T/C	0.19	0.22	2.10E-06	0.83(0.76-0.89)	----------
rs118167891	10	32946009	T/C	0.02	0.02	2.17E-06	1.93(1.47-2.53)	++++-++++++-?++-+-+--
rs6428588	1	90825206	T/C	0.30	0.34	2.69E-06	0.85(0.79-0.91)	----+--+---------+---
rs7982251	13	28909835	T/C	0.85	0.87	2.76E-06	0.79(0.72-0.87)	-------+--------+-++-
rs67658161	8	3286733	A/C	0.48	0.44	3.27E-06	1.16(1.09-1.24)	+++++++-+++-++++++++-+-
rs7979008	12	47479528	A/C	0.31	0.34	4.18E-06	0.85(0.79-0.91)	---------+-----+-+-+-
rs55893662	19	2955759	T/C	0.11	0.10	5.22E-06	1.46(1.24-1.73)	+-++--++++---++-+++++
rs5016373	15	34314041	T/C	0.61	0.65	6.28E-06	0.86(0.80-0.92)	---------+++----++-+-
rs190572487	14	19675351	T/C	0.75	0.73	6.86E-06	1.35(1.18-1.54)	-+-++++++++++-++++++
rs3847511	11	91454034	T/G	0.08	0.06	7.70E-06	1.36(1.19-1.55)	-++++++++++--+++--+--
rs165774	22	19952561	A/G	0.33	0.30	8.78E-06	1.18(1.10-1.27)	+++++++++-+-+++-++-+-
chr10_7228436_I	10	7228436	I2/D	0.14	0.12	9.25E-06	1.31(1.16-1.47)	-++------+-----+---++
rs12639760	4	135958271	A/T	0.22	0.19	9.31E-06	1.21(1.11-1.31)	+++++++-+++++++++++++
rs141199126	11	29761274	T/C	0.02	0.01	9.43E-06	2.05(1.49-2.83)	+?-++++++++?+?+-?+--+
chr7_123745464_I	7	123745464	I2/D	0.08	0.07	9.54E-06	1.32(1.17-1.49)	++--+----------
chr1_172543621_I	1	172543621	I2/D	0.03	0.04	1.02E-05	0.60(0.47-0.75)	++-++++++++-+++++++++
rs12799429	11	8097070	T/C	0.54	0.56	1.04E-05	0.86(0.80-0.92)	-+----+-----+-----++-
rs17077064	18	64976306	T/C	0.83	0.80	1.08E-05	1.21(1.11-1.31)	++-++++++++++++-+-++
rs117018753	13	110581806	T/C	0.07	0.06	1.12E-05	1.37(1.19-1.58)	+++-++++++++++-++-++

CHR, chromosome; BP, basepair position; freq, frequency; OR, odds ratio; CI, confidence interval

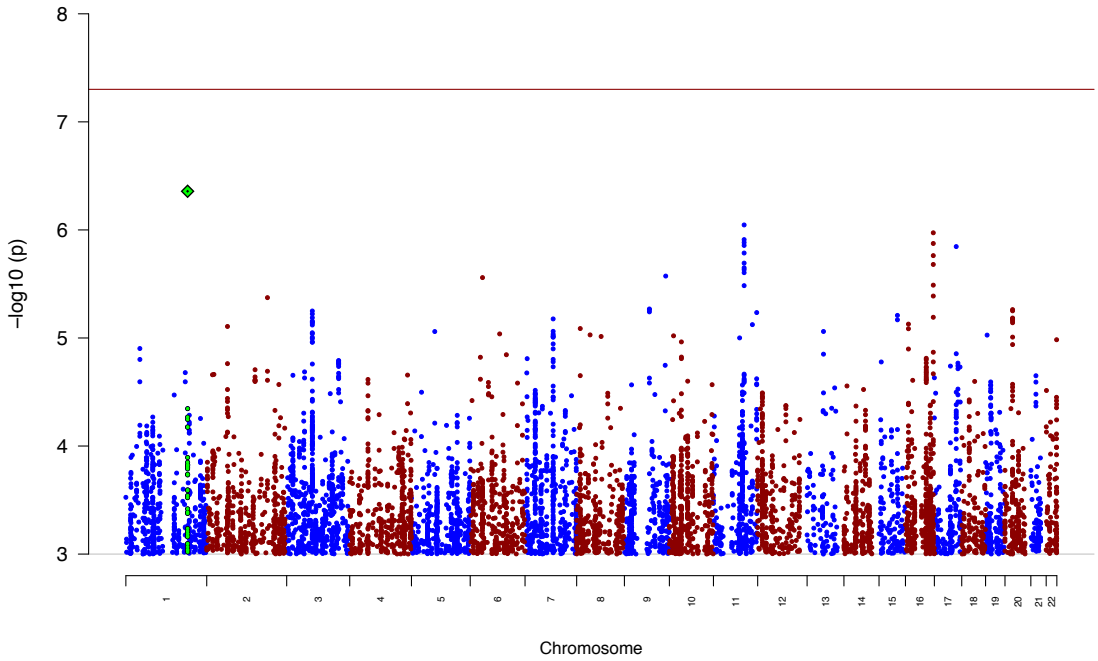

Figure S3: Manhattan plot of suicide attempt in schizophrenia

Table S10: Top 20 results from GWAS of suicide attempt in schizophrenia showing the most significant SNP from each genomic region

Variant	CHR	BP	A1/A2	A1 freq attempters	A1 freq non-attempters	P value	OR (C.I.)	Direction in each cohort
rs482039	1	190777567	T/C	0.03	0.02	4.39E-07	2.37(1.70-3.31)	+++-+++++
rs3858375	11	95077167	T/C	0.07	0.05	8.99E-07	1.60(1.33-1.94)	++++-++++
rs4843180	16	86753070	T/C	0.44	0.39	1.06E-06	1.25(1.14-1.36)	+++++++++
rs180697792	17	67294430	A/G	0.23	0.20	1.43E-06	1.34(1.19-1.50)	+++++++-++
rs72756712	9	128902906	A/G	0.93	0.95	2.67E-06	0.60(0.49-0.74)	---------
rs191312301	6	38975727	A/C	0.86	0.89	2.76E-06	0.71(0.62-0.82)	--+------
chr2_188481671_D	2	188481671	D/I3	0.34	0.38	4.23E-06	0.79(0.71-0.87)	---------
rs73650494	9	78831443	T/C	0.96	0.98	5.39E-06	0.56(0.43-0.72)	---------
rs6114731	20	24427180	A/G	0.04	0.03	5.46E-06	1.78(1.39-2.27)	+++++-+++
rs75305337	3	84333133	A/C	0.12	0.09	5.63E-06	1.39(1.20-1.60)	+++++++++
chr11_133764404_I	11	133764404	I5/D	0.79	0.75	5.82E-06	1.29(1.15-1.43)	------+--
rs57729539	15	78524199	A/G	0.80	0.83	6.18E-06	0.76(0.68-0.86)	---+-----
rs875777	7	86745380	T/C	0.16	0.20	6.66E-06	0.76(0.67-0.86)	--+------
rs6497871	16	10364163	A/G	0.64	0.60	7.44E-06	1.24(1.13-1.37)	+++++++++
rs151336980	11	120470737	T/C	0.04	0.03	7.52E-06	1.78(1.38-2.29)	++++-++-+
rs4494728	2	65589513	T/C	0.50	0.55	7.82E-06	0.82(0.75-0.89)	---------
rs2739958	8	12232534	T/C	0.58	0.60	8.18E-06	0.68(0.57-0.80)	--+----+-
rs73215273	13	72403250	A/C	0.88	0.90	8.71E-06	0.66(0.54-0.79)	-+-------
rs11739808	5	72745041	A/G	0.03	0.02	8.72E-06	2.30(1.59-3.31)	++-++++++
rs13198361	6	91673413	T/C	0.11	0.14	9.17E-06	0.65(0.54-0.79)	----+----

CHR, chromosome; BP, basepair position; freq, frequency; OR, odds ratio; CI, confidence interval

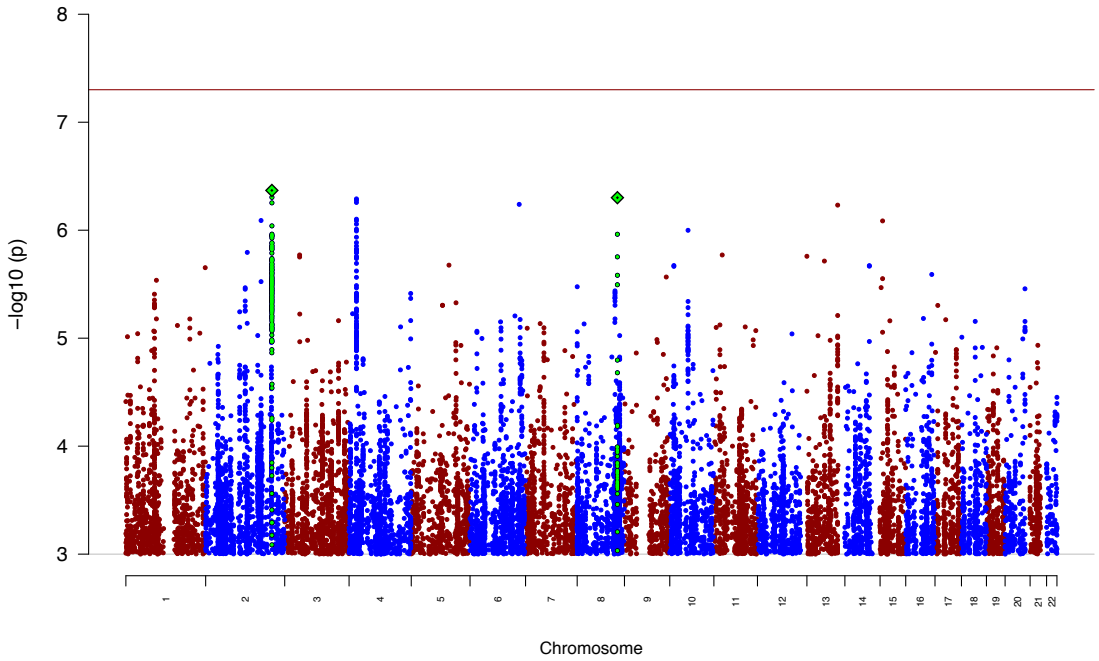

Figure S4: Manhattan plot of meta-analysis of suicide attempt in major depressive disorder, bipolar disorder and schizophrenia

Table S11: Top 20 results from meta-analysis of suicide attempt in MDD, BIP and SCZ showing the most significant SNP from each genomic region

Variant	CHR	BP	A1/A2	A1 freq attempters	A1 freq non-attempters	P value	OR (C.I.)	Direction in each cohort
rs149268645	2	203833018	A/G	0.14	0.15	4.28E-07	0.85(0.80-0.90)	---
rs4870888	8	125108977	T/C	0.52	0.54	5.00E-07	0.89(0.86-0.93)	---
chr4_23273116_D	4	23273116	D/I	0.19	0.18	5.13E-07	1.16(1.09-1.23)	+++
rs141252918	6	151828058	A/G	0.02	0.01	5.76E-07	1.73(1.39-2.14)	++?
rs9577511	13	113991823	A/G	0.86	0.87	5.86E-07	0.83(0.77-0.89)	---
rs62173322	2	170611029	A/G	0.86	0.87	8.13E-07	0.84(0.78-0.90)	---
rs76371172	15	31814455	T/G	0.98	0.98	8.20E-07	0.61(0.50-0.74)	---
rs11004733	10	56849344	T/C	0.04	0.04	1.00E-06	1.34(1.19-1.50)	+++
rs138689899	2	128288162	T/C	0.02	0.02	1.61E-06	1.53(1.29-1.82)	++-
rs142055939	3	45995554	T/C	0.02	0.02	1.69E-06	1.48(1.26-1.75)	+++
rs35107435	11	27249330	A/T	0.16	0.15	1.70E-06	1.17(1.10-1.24)	+++
rs113988902	13	19525105	T/C	0.05	0.04	1.74E-06	1.52(1.28-1.80)	?++
chr13_73243177_I	13	73243177	I/D	0.04	0.03	1.93E-06	1.59(1.32-1.93)	?--
rs186672572	5	116878032	T/C	0.02	0.01	2.11E-06	1.64(1.33-2.00)	+++
rs113386487	10	13358583	A/T	0.98	0.98	2.12E-06	0.67(0.56-0.79)	---
rs73348245	14	96158072	A/G	0.06	0.06	2.13E-06	1.27(1.15-1.40)	+++
rs6426297	1	246538381	T/C	0.02	0.01	2.22E-06	1.65(1.34-2.04)	+++
rs73577700	16	80280761	A/T	0.83	0.85	2.57E-06	0.87(0.82-0.92)	---
rs72756712	9	128902906	A/G	0.94	0.95	2.71E-06	0.78(0.70-0.86)	---
rs75633108	1	96735185	T/C	0.02	0.02	2.91E-06	1.59(1.31-1.93)	+++

MDD, major depressive disorder; BIP, bipolar disorder; SCZ, schizophrenia; CHR, chromosome; BP, basepair position; OR, odds ratio; CI, confidence interval

Table S12: Top 20 results from meta-analysis of suicide attempt in mood disorders showing the most significant SNP from each genomic region

Variant	CHR	BP	A1/A2	A1 freq attempters	A1 freq non-	P value	OR (C.I.)	Direction in each cohort
rs138689899	2	128288162	T/C	0.02	0.01	**2.50E-08**	1.75(1.44-2.14)	++
rs28591567	4	23253912	A/G	0.78	0.80	**3.11E-08**	0.84(0.79-0.89)	--
chr6_151835609_D	6	151835609	I/D	0.94	0.95	3.66E-07	0.74(0.66-0.83)	++
chr13_61834504_D	13	61834504	I/D	0.91	0.92	5.77E-07	0.78(0.71-0.86)	++
rs186672572	5	116878032	T/C	0.02	0.01	7.19E-07	1.78(1.42-2.24)	++
rs112944737	8	134677667	T/C	0.91	0.92	1.11E-06	0.80(0.73-0.87)	--
rs9577511	13	113991823	A/G	0.86	0.87	1.35E-06	0.81(0.75-0.88)	--
rs150795632	6	37439376	A/G	0.98	0.98	1.69E-06	0.59(0.48-0.73)	--
rs113051785	11	20167807	C/G	0.02	0.01	1.70E-06	1.75(1.39-2.20)	++
rs1355048	1	90830490	T/C	0.37	0.40	1.91E-06	0.88(0.84-0.93)	--
rs115833694	4	99918226	C/G	0.98	0.98	1.99E-06	0.63(0.52-0.76)	--
rs17764923	6	159820779	A/G	0.16	0.14	2.04E-06	1.19(1.11-1.27)	++
rs117020391	12	107099751	T/C	0.98	0.98	2.09E-06	0.63(0.52-0.76)	--
rs72832403	2	115551269	A/G	0.92	0.93	2.16E-06	0.79(0.72-0.87)	--
rs150320200	4	23450330	A/G	0.97	0.98	2.19E-06	0.66(0.55-0.78)	--
rs114598476	1	224438315	A/G	0.96	0.97	2.30E-06	0.67(0.57-0.79)	--
rs76400344	22	26697120	A/C	0.03	0.02	3.01E-06	1.52(1.28-1.82)	++
chr2_124851208_D	2	124851208	I/D	0.96	0.96	3.48E-06	0.72(0.62-0.82)	++
rs143457262	7	82072544	A/G	0.03	0.03	4.16E-06	1.51(1.26-1.79)	++
rs3829881	1	117531813	T/C	0.49	0.47	4.45E-06	1.12(1.07-1.18)	++

CHR, chromosome; BP, basepair position; freq, frequency; OR, odds ratio; CI, confidence interval

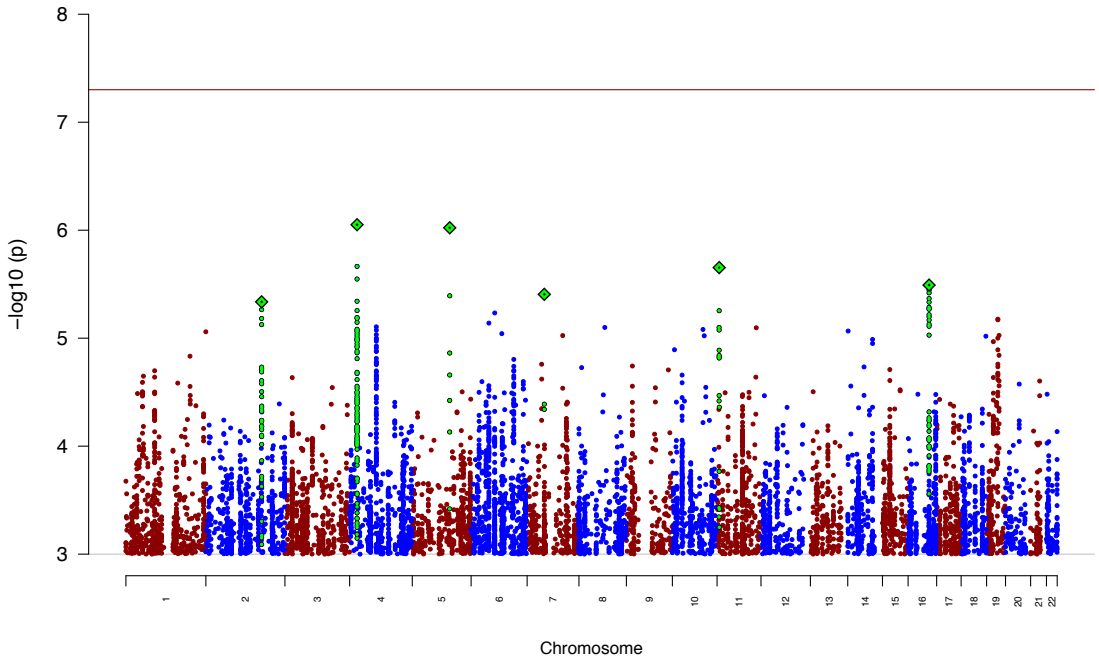

Figure S5: Manhattan plot of meta-analysis of suicide attempt in bipolar disorder and schizophrenia

Table S13: Top 20 results from meta-analysis of suicide attempt in BIP and SCZ showing the most significant SNP from each genomic region

Variant	CHR	BP	A1/A2	A1 freq attempters	A1 freq non-	P value	OR (C.I.)	Direction in each cohort
chr4_23273116_D	4	23273116	D/I	0.19	0.17	8.90E-07	1.19(1.11-1.27)	++
rs26318	5	115687905	T/C	0.98	0.99	9.50E-07	0.52(0.40-0.67)	--
rs118102650	11	9099847	A/G	0.02	0.01	2.22E-06	1.79(1.41-2.28)	++
rs12925656	16	65107920	T/C	0.88	0.90	3.22E-06	0.82(0.76-0.89)	--
rs73122740	7	53695512	T/G	0.92	0.91	3.93E-06	1.26(1.14-1.39)	++
rs2353181	2	170305256	T/C	0.95	0.94	4.61E-06	1.33(1.17-1.49)	++
rs75237141	6	72966596	A/G	0.90	0.92	5.84E-06	0.80(0.73-0.88)	--
rs56342621	19	35071781	C/G	0.98	0.99	6.67E-06	0.61(0.49-0.75)	--
rs9475195	6	55061018	T/C	0.58	0.61	7.25E-06	0.89(0.84-0.93)	--
chr4_82106147_I	4	82106147	I/D	0.07	0.06	7.85E-06	1.29(1.15-1.44)	--
chr8_80591790_D	8	80591790	I/D	0.61	0.63	7.95E-06	0.88(0.83-0.93)	++
rs10892827	11	122210990	T/G	0.90	0.92	8.01E-06	0.80(0.73-0.88)	--
rs189924441	10	94182243	A/G	0.97	0.97	8.30E-06	0.67(0.56-0.80)	--
rs8022689	14	21608986	A/G	0.38	0.35	8.58E-06	1.14(1.07-1.20)	++
rs3007305	1	246862572	C/G	0.66	0.68	8.72E-06	0.88(0.83-0.93)	--
rs72924216	6	94570858	A/G	0.95	0.96	9.08E-06	0.72(0.63-0.83)	--
rs66666015	19	38717143	T/C	0.76	0.74	9.42E-06	1.16(1.09-1.24)	++
rs38758	7	109943767	A/C	0.47	0.44	9.46E-06	1.12(1.07-1.18)	++
rs117559494	10	98008526	A/G	0.03	0.03	9.50E-06	1.53(1.27-1.85)	++
rs117637007	18	75827605	T/C	0.11	0.13	9.58E-06	0.80(0.72-0.88)	--

CHR, chromosome; BP, basepair position; freq, frequency; OR, odds ratio; CI, confidence interval

Table S14: Comparison of genome-wide significant locus on chr4 between GWAS on suicide attempt, MDD, BIP and SCZ

GWAS (References)	CHR	Variant	BP	A1	P value	OR (C.I.)
SA in MDD	4	rs28591567	23253912	G	0.030	1.11(1.05-1.18)
SA in BIP	4	rs28591567	23253912	G	6.78E-08	1.25(1.15-1.35)
SA in SCZ	4	rs28591567	23253912	G	0.674	1.02(0.96-1.09)
MDD 1	4	rs28591567	23253912	G	0.010	0.98(0.97-1.00)
BIP 2	4	rs28591567	23253912	G	0.231	0.98(0.95-1.01)
SCZ 3	4	rs28591567	23253912	G	0.038	0.97(0.95-1.00)

MDD, major depressive disorder; BIP, bipolar disorder; SCZ, schizophrenia; SA, suicide attempt

References

Number Citation

1 Major Depressive Disorder Working Group of the Psychiatric Genomics Consortium, Genome-wide association analyses identify 44 risk variants and refine the genetic architecture of major depression. bioRxiv, (2017).

2 Bipolar Disorder Working Group of the Psychiatric Genomics Consortium, unpublished data from GWAS of 20352 bipolar disorder cases and 31358 controls.

3 Schizophrenia Working Group of the Psychiatric Genomics Consortium, Biological insights from 108 schizophrenia-associated genetic loci. Nature, 511, 421-7 (2014).

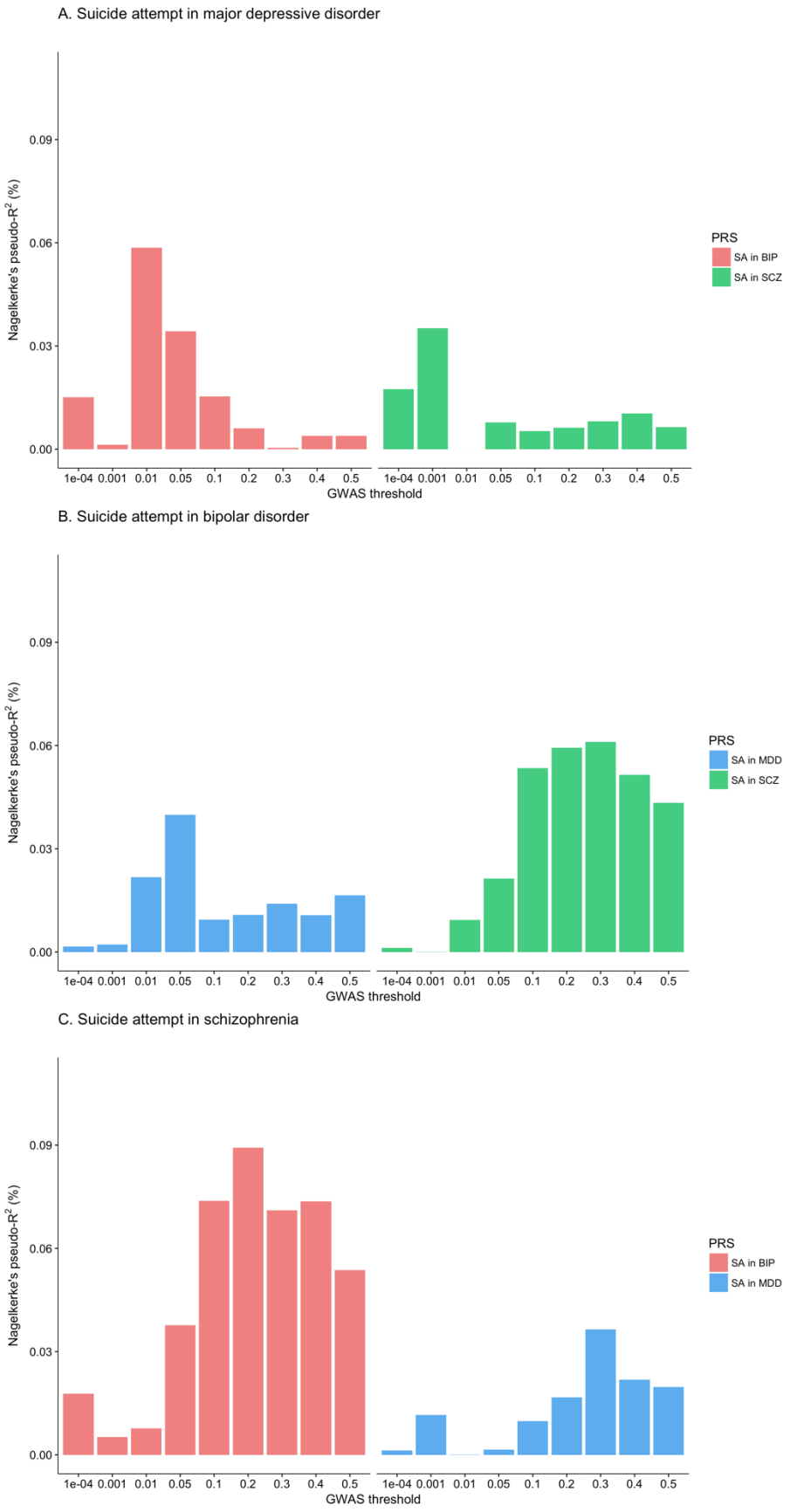

Figure S6: Polygenic risk scores for suicide attempt used to predict suicide attempt in A - major depressive disorder, B - bipolar disorder and C - schizophrenia.
PRS-polygenic risk score, SA-suicide attempt, MDD-major depressive disorder, BIP-bipolar disorder, SCZ-schizophrenia.

6. Investigation of blood mRNA biomarkers for suicidality in an independent sample

This chapter is presented as a published paper and is an exact copy of the following journal publication:

MULLINS, N., HODGSON, K., TANSEY, K. E., PERROUD, N., MAIER, W., MORS, O., RIETSCHEL, M., HAUSER, J., HENIGSBERG, N., SOUERY, D., AITCHISON, K., FARMER, A., MCGUFFIN, P., BREEN, G., UHER, R. & LEWIS, C. M. 2014. Investigation of blood mRNA biomarkers for suicidality in an independent sample. *Transl Psychiatry,* 4**,** e474.

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ORIGINAL ARTICLE

Investigation of blood mRNA biomarkers for suicidality in an independent sample

N Mullins[1], K Hodgson[1], KE Tansey[2], N Perroud[3], W Maier[4], O Mors[5], M Rietschel[4,6], J Hauser[7], N Henigsberg[8], D Souery[9,10], K Aitchison[1,11], A Farmer[1], P McGuffin[1], G Breen[1,12], R Uher[1,13] and CM Lewis[1,14]

Changes in the blood expression levels of *SAT1, PTEN, MAP3K3* and *MARCKS* genes have been reported as biomarkers of high versus low suicidality state (Le-Niculescu *et al.*). Here, we investigate these expression biomarkers in the Genome-Based Therapeutic Drugs for Depression (GENDEP) study, of patients with major depressive disorder on a 12-week antidepressant treatment. Blood gene expression levels were available at baseline and week 8 for patients who experienced suicidal ideation during the study ($n = 20$) versus those who did not ($n = 37$). The analysis is well powered to detect the effect sizes reported in the original paper. Within either group, there was no significant change in the expression of these four genes over the course of the study, despite increasing suicidal ideation or initiation of antidepressant treatment. Comparison of the groups showed that the gene expression did not differ between patients with or without treatment-related suicidality. This independent study does not support the validity of the proposed biomarkers.

Translational Psychiatry (2014) **4,** e474; doi:10.1038/tp.2014.112; published online 28 October 2014

INTRODUCTION

Suicide is a worldwide public health problem and is among the ten leading causes of death.[1] Suicidal ideation is a risk factor for suicidal behavior, but its assessment has to rely on imprecise and subjective measures, hampered by patients' reluctance to report suicidal thoughts.[2,3] While many clinical variables are correlated with suicidality, they are insufficient to identify risk in individual patients.[3,4] Objectively measured biomarkers could contribute to better risk prediction and clinical care.

A recent study by Le-Niculescu *et al.*[5] investigated biomarkers for suicidal ideation in a live discovery sample of patients with bipolar disorder ($n = 9$). A Convergent Functional Genomics approach was used to prioritize genes which were differentially expressed between a high versus low suicidality state, on the basis of findings from postmortem brain gene expression studies of suicide victims, as well as genetic linkage or association studies on suicide. The top biomarkers were tested for differential expression in a validation sample of suicide victims ($n = 9$) and for ability to predict past and future hospitalizations for suicidality in two follow-up cohorts with either bipolar disorder ($n = 42$) or psychosis ($n = 46$). It was reported that changes in the expression of four genes: spermidine/spermine N1-acetytransferase 1 (*SAT1*), phosphatase and tensin homolog (*PTEN),* mitogen-activated protein kinase kinase kinase 3 (*MAP3K3*) and myristoylated alanine-rich protein kinase C substrate (*MARCKS)* in the blood, could be used as biomarkers of a high versus low suicidal state and could predict hospitalizations for suicidality.[5]

Biomarker research, in general, is plagued with overestimation of results in discovery studies with subsequent lack of replication, and findings which usually have limited predictive ability.[6,7] Currently, in psychiatry there are no biomarkers of clinical utility.[7] In suicidality biomarker research, the study of genetic, immunological and neuroendocrine biomarkers has generated inconsistent results, with little or no replication of initial findings.[8] Replication in large independent samples by independent research groups is essential to validate the results of biomarker discovery studies. Here, we investigate the expression of the proposed biomarkers *SAT1, PTEN, MAP3K3* and *MARCKS,* in patients with depression who experienced suicidal ideation during antidepressant treatment.

MATERIALS AND METHODS

Sample collection

The Genome-Based Therapeutic Drugs for Depression Study (GENDEP) is a prospective pharmacogenetic study of patients with major depressive disorder ($n = 868$) receiving 12-week antidepressant treatment.[9] Participants were recruited from nine European centers and diagnosed with major depressive disorder using the Schedules for Clinical Assessment in Neuropsychiatry Interview (SCAN), according to the International Classification of Diseases 10th edition (ICD-10) or Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV).[10–12] SCAN interviews were conducted by psychologists or psychiatrists trained at World Health Organisation Training and Research Centres. Exclusion criteria were psychotic disorder with mood incongruent psychotic symptoms or bipolar

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disorder.[9] Patients received a protocol-guided treatment with either escitalopram—a selective serotonin reuptake inhibiter or nortriptyline—a tricylic antidepressant.[9] All participants in the GENDEP study were of Caucasian European parentage.[9]

Patients gave written informed consent and ethical approval was obtained from the local ethics committee at each center of recruitment. The GENDEP trial is registered at EudraCT (no. 2004-001723-38) and ISRCTN (no. 03693000).

Gene expression measurement

Blood was collected in PAXgene tubes (PreAnalytiX, Hombrechtikon, Switzerland) at both week 0 and week 8 for 136 participants and frozen at −80 °C.[13] PAXgene tubes were allowed to thaw for 12 h at room temperature and mRNA was isolated from whole blood using the Qiagen PAXgene Blood miRNA Kit (PreAnalytiX) following the manufacturer's protocol.[14] Genome-wide expression analysis was performed in four batches on Illumina Human HT-12 v4 BeadChip microarrays (Illumina, San Diego, CA, USA).

Quality control was performed using R 3.0.2. Gene expression values were log transformed. In the analysis of outliers of gene expression, 13 patients were excluded because the expression in one of their paired samples fell below 2 s.d. from the mean inter-array correlation. Additional filtering using sex-incongruent expression of probes within the *XIST* gene removed a further two samples. Detection score *P*-values were used for probe filtering ($P < 0.1$ in at least one sample), and probes displaying little variation were also removed (where s.d. was in the lowest quartile < 0.12030). After filtering, a total of 121 paired samples remained, with 29 765 probes. Data were normalized using quantile normalization, and ComBat was used to control for batch effects.[15] Probes of interest were ILMN_1753342 (*SAT1*), ILMN_1701134 (*PTEN*), ILMN_1779010 and ILMN_2296697 (mean expression level was used for *MAP3K3*) and ILMN_1807042 (*MARCKS*).

Phenotype definition

Suicidal ideation was assessed weekly using items from the clinician-rated 17-item Hamilton Rating Scale for Depression, the Montgomery-Åsberg Depression Rating Scale and the self-report Beck Depression Inventory.[16–18] Response options of these items are shown in Table 1.

The three items were combined into a composite suicidal ideation score using item response theory.[19] Significant suicidal ideation at baseline was defined as at least 1 s.d. above the minimum score on the composite scale.[19] As previously described, treatment-worsening suicidal ideation was considered an increase of at least 0.5 s.d., above their original score in a patient with significant suicidal ideation at baseline. Treatment-emergent suicidal ideation was defined as surpassing the threshold for suicidal ideation and an increase of 0.5 s.d. above their original score, in patients without significant suicidal ideation at baseline.[19] Individuals with either treatment-emergent suicidal ideation or treatment-worsening suicidal ideation at any point during the 12-week study were used as cases of treatment-related suicidal ideation (RxSI+; $n = 20$). The worst week for suicidal ideation emerging or increasing was week 5 and none of the patients became suicidal or worsened after week 8.[19] This definition corresponds to an increase of one unit on the Hamilton Rating Scale for Depression, as used by Le-Niculescu *et al*.[5] Individuals with scores under the threshold for suicidal ideation at each week and who did not show an increase of > 0.5 s.d. above their baseline score were used as controls (non-SI; $n = 37$).[19] The remaining individuals with paired transcriptomics data were excluded as they did not meet this case or control definition ($n = 64$).

Table 1. Range of response options for HRSD-17, MADRS and BDI suicide items

Scale	*Score*	*Meaning*
HRSD-17	0	Absent
	1	Feels life is not worth living
	2	Wishes he/she were dead, or any thought of possible death to self
	3	Suicide ideas or half-hearted attempt
	4	Attempts suicide
MADRS	0–1	Enjoys life or take it as it comes
	2–3	Weary of life. Only fleeting suicidal thoughts
	4–5	Probably better off dead. Suicidal thoughts are common, and suicide is considered as a possible solution, but without specific plans or intentions
	6	Explicit plans for suicide when there is an opportunity. Active preparation of suicide
BDI	0	Absent
	1	Thought of killing myself
	2	I would like to kill myself
	3	I would like to kill myself if I had a chance

Abbreviations: BDI, Beck Depression Inventory; HRSD, Hamilton Rating Scale for Depression; MADRS, Montgomery–Åsberg Depression Rating Scale.

Statistical analysis

Two analyses were used to test for change in expression in RxSI+ patients and control (non-SI) patients. In a within-subjects design, a paired sample *t*-test was used to compare gene expression at week 0 and week 8 within the case and control groups, following the protocol used by Le-Niculescu *et al*.[5] In a between-subjects design, the relationship between case (RxSI+) and control (non-SI) status and change in gene expression (week 8 − week 0) was assessed using logistic regression, co-varying for age, sex, drug treatment, gene expression at week 0 and also center of recruitment, to capture any remaining variation in population structure.

Power calculation

This study had 98% power to detect a standardized difference in expression of 1.14 between cases with suicidal ideation versus non-suicidal controls, the largest reported difference in gene expression in the original paper.[5] There was also good power to detect smaller changes in gene expression between cases and controls, with 80% power to detect an effect size of 0.79 and 60% power to detect an effect size of 0.62.

RESULTS

Individuals with suicidal ideation were significantly older than controls ($P = 0.02$), whereas there was no difference in sex or drug treatment between suicidal ideation cases and controls (Table 2).

Within-subjects comparison

No significant difference in expression between week 0 and week 8 was detected for any gene, within either the RxSI+ cases or the non-SI controls (Table 3). Further, initiation of antidepressant treatment had no effect on the expression of these four genes in RxSI+ cases and non-SI controls (Figure 1).

Between-subjects comparison

The change in gene expression from week 0 to week 8 was compared between RxSI+ cases and non-SI controls using logistic regression, co-varying for age, sex, drug treatment, gene

Table 2. Characteristics of the GENDEP suicidal ideation sample

	RxSI+ cases (n = 20) (%)	*Non-SI controls (n = 37) (%)*	P-*value*
Sex			1.000
Male	5 (25.0%)	10 (27.0%)	
Female	15 (75.0%)	27 (72.9%)	
Mean age (years) (s.d.)	48.7 (13.3)	39.8 (12.8)	0.020
Drug			0.779
Escitalopram	12 (60.0%)	25 (67.6%)	
Nortriptyline	8 (40.0%)	12 (32.4%)	

Abbreviations: non-SI, controls without suicidal ideation; RxSI+, treatment-related suicidal ideation. *P*-value was determined using a chi-squared test, with the exception of age, where a nonparametric Mann–Whitney *U*-test was used.

expression at week 0 and center of recruitment. There was no significant difference in the change in gene expression between cases versus controls for any gene tested (Table 4).

DISCUSSION

Suicidal ideation is difficult to predict and assess, so the use of objectively measured biomarkers would be advantageous. Contrary to the findings of Le-Niculescu *et al*.,[5] our analysis of the blood expression levels of *SAT1*, *PTEN*, *MAP3K3* and *MARCKS* genes showed no difference between depressed patients with suicidal ideation versus those without.

Although the sample size in this study is small ($n = 20$ cases and $n = 37$ controls), it is larger than the primary analysis, which used a discovery cohort of nine patients and three small and heterogeneous replication samples.[5] The original analysis was conducted in an all-male sample and so results may lack generalizability. The GENDEP sample is mixed and thus more representative of the natural epidemiology of suicidality in major depressive disorder.[20,21] The index of suicidal ideation used here incorporates three clinical scales (including that used in the original report) with a mixture of patient self-report and clinician ratings. The within-subjects analysis used to compare gene expression at week 0 and week 8 is a powerful design as it can remove the possible influence of genetics, as well as other patient-specific factors, on suicidal ideation during the study period.[22,23] Furthermore, this study has 98% power to detect the effect sizes previously reported.

Expression of *SAT1* in our study was slightly lower in RxSI+ cases than in non-SI controls, though not significantly different. This is in the opposite direction to the findings reported by Le-Niculescu *et al*.,[5] though in support of previous studies, which demonstrated decreased levels of *SAT1* mRNA in several brain regions of suicide victims.[24–28]

Assessment of blood biomarkers may not be a reliable representation of brain function but it does provide easily

Table 3. Difference in gene expression between week 0 and week 8

Gene	RxSI+ cases		Non-SI controls	
	Mean difference in expression (s.d.)	P-value	Mean difference in expression (s.d.)	P-value
SAT1	−0.072 (0.311)	0.312	0.018 (0.196)	0.570
PTEN	−0.037 (0.361)	0.648	−0.014 (0.365)	0.817
MAP3K3	0.023 (0.194)	0.591	0.009 (0.269)	0.834
MARCKS	−0.148 (0.353)	0.075	−0.003 (0.306)	0.950

Abbreviations: non-SI, controls without suicidal ideation; RxSI+, treatment-related suicidal ideation. *P*-values were calculated using a paired *t*-test.

Table 4. Difference in change in gene expression between week 0 and week 8 in cases versus controls

Gene	Regression coefficient	Standard error	P-value
SAT1	−2.067	1.707	0.226
PTEN	−0.737	1.265	0.560
MAP3K3	−2.326	2.541	0.360
MARCKS	−2.240	1.401	0.110

P-values were calculated using a logistic regression controlling for age, sex, drug, expression at week 0 and center of recruitment.

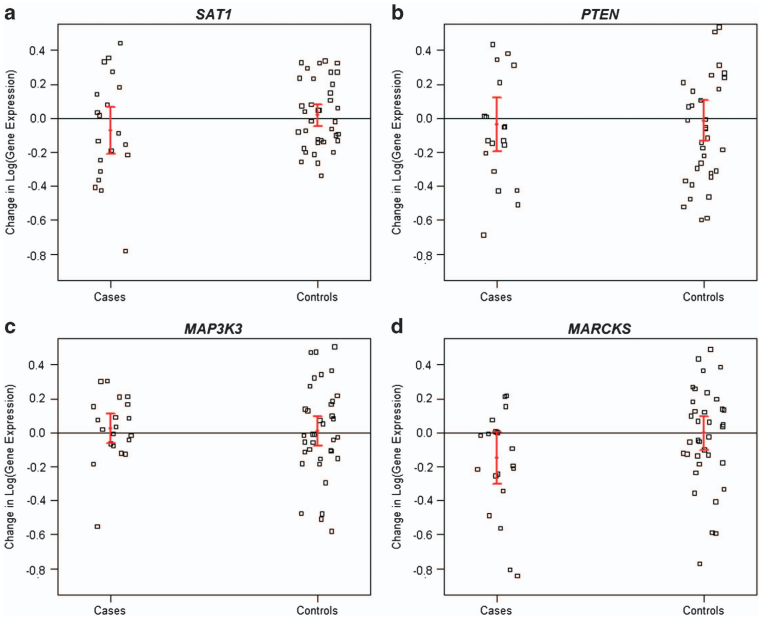

Figure 1. (**a**–**d**) Change in gene expression (week 8 – week 0) in RxSI+ case and non-SI control groups for (**a**) SAT1, (**b**) PTEN, (**c**) MAP3K3 and (**d**) MARCKS. Error bars represent 1 s.e.m. change in expression. RxSI+, treatment-related suicidal ideation; non-SI, controls without suicidal ideation.

obtainable measures which could be useful in patient monitoring. Suicidal ideation is a complex phenotype and its etiology is poorly understood. It is likely that larger sample sizes and a model including multiple clinical and biological risk factors, will be required to form a robust predictor with clinical utility.

CONFLICT OF INTEREST

AF and PM have received consultancy fees and honoraria for participating in expert panels for pharmaceutical companies, including GlaxoSmithKline. CML has received consultancy honoraria from Eli Lilly. The remaining authors declare no conflict of interest.

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7. Discussion

7.1 Summary of findings

The aim of this thesis was to dissect the genetic and environmental heterogeneity of MDD using novel approaches in the largest available clinical and population samples. Chapter 2 began with a state-of-science review on the challenges that have faced depression genetics and the strategies that have been used by recent successful GWAS, such as targeted efforts to reduce heterogeneity in depression and innovative approaches to increase sample size (Mullins and Lewis, 2017).

Chapter 3 tested interactions between polygenic risk scores for MDD and environmental adversity in the Radiant UK recurrent depression sample (Mullins et al., 2016). This study found no interaction between PRS for MDD and adult stressful life events, but significant correlation between them. Evidence of interaction was found between PRS for MDD and childhood trauma, but showed an inverse association with depression, as individuals with high childhood trauma scores tended to have a lower genetic liability for depression.

To investigate how psychiatric disorders persist, despite the reduced fecundity of affected individuals, Chapter 4 examined the selection pressures on common and rare genetic variants for psychiatric disorders in unaffected individuals in the Icelandic population (Mullins et al., 2017). PRS for attention deficit hyperactivity disorder (ADHD) were associated with having more children, while PRS for autism were associated with having fewer children and later age at first child. Rare copy number variants (CNVs) implicated in autism and schizophrenia were associated with having fewer children, in carriers who are unaffected by psychiatric illness.

Chapter 5 presented the largest GWAS on suicide attempt to date, including over 6,000 attempters and 17,000 non-attempters with MDD, bipolar disorder and schizophrenia, recruited from the Psychiatric Genomics Consortium. The study found three genome-wide significant loci for suicide attempt in MDD or bipolar disorder, which will be tested in independent replication samples. This is the first consortium-based GWAS on suicide attempt and makes progress in amassing the large sample sizes required for robust genetic studies on this serious condition. Finally, in Chapter 6, an independent test of blood mRNA levels of *SAT1, PTEN, MAP3K3* and *MARCKS* in the GENDEP study, provided no support for these proposed biomarkers for suicidality (Mullins et al., 2014a). Here, I will discuss the findings of the thesis more broadly and place them in the context of the current literature.

7.2 Depression GWAS

After years of lagging behind other psychiatric disorders, GWAS on depression are now beginning to identify the genetic variants involved (Mullins and Lewis, 2017). The CONVERGE consortium achieved this through systematic efforts to reduce heterogeneity, focusing on Chinese women with recurrent severe MDD (CONVERGE consortium, 2015). Studies conducted by the Social Science Genetic Association Consortium (SSGAC) and in collaboration between the PGC and CHARGE Consortia, combined samples with clinical diagnosis of MDD and measures of depressive symptoms, breaking down the boundary between disease state and normal variation in mood (Okbay et al., 2016, Direk et al., 2016). In a novel approach to increase sample size, 23andMe used self-reported MDD data from their online consumers to collect large numbers of cases and controls (Hyde et al., 2016). More recent unpublished work by the PGC has identified 44 independent loci for depression in a sample size of over 130,000 cases and 330,000 controls, combining clinically ascertained MDD samples, cases from population biobanks and the self-report data from 23andMe (Major Depressive Disorder Working Group of the Psychiatric Genomics Consortium et al., 2017). This study has made rapid progress in increasing sample size, achievable since depression is a common disorder, and MDD now has the largest number of cases of any disorder investigated within the PGC (Sullivan et al., 2017).

GWAS on depression have amassed the critical sample size necessary to reach an inflection point, beyond which the number of genetic associations is predicted to increase linearly with sample size, as seen for other disorders (Levinson et al., 2014). There are a number of future directions for this research. First, more genetic associations remain to be found. Several studies have shown that the genetic correlation between MDD and measures of depressive symptoms in general population samples is very high (Major Depressive Disorder Working Group of the Psychiatric Genomics Consortium et al., 2017, Okbay et al., 2016, Direk et al., 2016). This means that future studies should be able to elucidate the bulk of the common variant genetic architecture of MDD using a cost-effective shortcut - large studies of genotyped individuals who complete brief lifetime MDD screening as opposed to lengthy and expensive clinical interviews. This strategy can be used to identify genetic associations which can then be followed up in smaller samples with more detailed phenotypic information. Second, once genome-wide associations have been found, a key challenge for depression and other diseases is to identify the causal risk variants within these linkage disequilibrium windows of association, which will provide information on the biological mechanisms of disease. Several lines of evidence indicate that the causal variants are more likely those that control gene expression. For example, GWAS hits are enriched for expression quantitative trait loci (eQTLs), which influence the expression level of one or more genes (Albert and Kruglyak, 2015). DNAse I hypersensitivity sites (DHS),

which regulate chromatin structure and gene transcription, have been shown to explain almost 80% of the h^2_{SNP} for eleven common diseases including bipolar disorder, compared with coding variants which explain less than 10% (Gusev et al., 2014). Novel analytical and bioinformatics methods which integrate multiple levels of "omic" data from different tissues, such as genomic, transcriptomic and epigenomic, are now being utilised to prioritise and interpret GWAS results for functional follow-up studies (Zhu et al., 2016, Gamazon et al., 2015, Gusev et al., 2016). The ultimate goal is to uncover the molecular mechanisms underlying these genetic associations and translate these into biomarkers for depression and novel targets for antidepressant drugs.

7.3 PRS-by-environment interactions

Although GWAS on depression are starting to make breakthroughs, environmental influences still play a crucial role and understanding the interplay between genes and environment could provide important insights into the disorder's aetiology. In Chapter 3 of the thesis, I showed lack of evidence for gene-by-environment interactions between PRS for MDD and adult stressful life events in the Radiant UK recurrent depression sample (Mullins et al., 2016). This is consistent with findings from the Health and Retirement Study, which examined this interaction in the context of depressive symptoms in older adults (Musliner et al., 2015). Both of these studies concluded that genetic liability and stressful life events combine additively to increase risk of depression. However, one limitation is that the PRS used were generated from results of the PGC mega-analysis of MDD, which had a modest sample size of approximately 9,000 cases and controls and was underpowered (Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium et al., 2013). More recently, another test of the PRS-by-stressful life event interaction has been conducted in a sample of 5,221 adults from the Australian Twin Registry (Colodro-Conde et al., 2017). This study used a more powerful PRS for MDD generated from the results of an intermediate discovery GWAS conducted by the PGC on a sample of 49,524 MDD cases and 110,074 controls. The study demonstrated an interaction between PRS and personal stressful life events contributing positively to depressive symptoms (Colodro-Conde et al., 2017). In contrast to previous results, this would be in line with a diathesis-stress model, which says that the effects of stress on risk of depression are dependent on underlying genetic susceptibility to the disorder (Monroe and Simons, 1991).

In Radiant UK, evidence of interaction between PRS for MDD and childhood trauma was found, but showed an inverse association with depression as individuals with high childhood trauma scores tended to have a lower genetic liability for depression (Mullins et al., 2016). The findings

were in the opposite direction to results from the NESDA study where a significant interaction with childhood trauma increased risk for depression (Peyrot et al., 2014). To further explore these findings, the PGC subsequently conducted a meta-analysis of interaction results across Radiant UK, NESDA and seven other MDD cohorts with childhood trauma information available, again using a more powerful MDD PRS (Peyrot et al., 2017). This analysis showed no evidence for interaction between PRS and childhood trauma. The study concluded that the previously reported interaction effects in Radiant UK and NESDA, although both statistically significant, can best be interpreted as chance findings and that risk for MDD is unlikely to be attributable to genome-wide moderation of genetic effects by childhood trauma.

The discrepant findings from these polygenic interaction studies have no obvious explanation. One source of heterogeneity in the studies of stressful life events is their timing, with Radiant UK focusing on the 6 months prior to the worst episode of depression (Mullins et al., 2016), and other studies measuring depressive symptoms and events in the previous year (Colodro-Conde et al., 2017), or two years (Musliner et al., 2015). Another possibility is that interaction effects are small and studies so far have been underpowered, increasing their statistical vulnerability to false findings. There is a need for larger MDD datasets with uniform environmental measures to facilitate direct replications and meta-analysis across studies. This is currently the major barrier in progressing GxE research, as opposed to genotyping samples which is comparatively easy and cheap.

Chapter 3 also showed gene-environment correlations (rGE) whereby PRS for MDD were associated with more stressful life events, specifically dependent events which are likely to be related to a person's own behaviour (Mullins et al., 2016). The h^2_{SNP} of stressful life events has previously been estimated at 30%, and this rGE with common variants for MDD has now been replicated by other studies (Dunn et al., 2016, Colodro-Conde et al., 2017, Power et al., 2013b). This suggests that individuals with genetic liability for MDD are more likely to select themselves into adversity, creating stressful life events or eliciting certain responses from others in their environment, which are known as active and evocative gene-environment correlations (Jaffee and Price, 2008).

One final consideration for polygenic interaction testing, is that all of the aforementioned studies have used a PRS based on SNPs which have a main effect on MDD in a GWAS blind to environmental exposures. Interactions may occur with individual SNPs or SNPs which do not have a main effect on MDD. Genome-wide gene-by-environment interaction studies (GWEIS) have begun to test interactions at individual SNPs in depression, although currently have limited

power as most large genotyped samples are missing adequate environmental data (Dunn et al., 2016, Otowa et al., 2016). In a similar approach, a GWAS on sensitivity to the environment has also been conducted (Keers et al., 2016). This novel design included one of each pair of monozygotic twins, with the outcome measure being the twins' intra-pair difference in emotional problems (Keers et al., 2016). The basic principle is that the variability in outcome between a monozygotic twin pair must be the result of non-shared environmental effects and genetic variants involved in sensitivity to these environments will increase the discordance between the twins. A PRS for environmental sensitivity generated from these results moderated the effects of positive and negative parenting on emotional problems in an independent sample and moderated the efficacy of different types of psychological treatment for anxiety (Keers et al., 2016). This represents a shift to focusing on the differential susceptibility model, which suggests that genotypes can increase sensitivity to environments, whether negative or positive, and individuals who are most vulnerable to adverse environments may also be those who reap the most benefit from positive ones (Belsky and Pluess, 2009). Studying positive environments is often neglected in psychiatric research and uncovering protective interactions could contribute to the development of intervention strategies. Both GWEIS and GWAS of environmental sensitivity are promising new experimental designs for dissecting the interplay between genes and environment, but leveraging the most from these methods will depend on the availability of environmental data.

7.4 Selection on genetic variants for psychiatric disorders

To investigate how psychiatric disorders persist, despite the reduced fecundity of affected individuals, Chapter 4 examined the selection pressures on common and rare genetic variants for psychiatric disorders in unaffected individuals in the Icelandic population (Mullins et al., 2017). PRS for ADHD were associated with having more children, while PRS for autism were associated with having fewer children, indicating that common genetic variants that are thousands of years old are currently subject to weak selection pressure. It is perhaps surprising that these two PRS were associated with fecundity, given that they have lower predictive power than others tested, for example the PRS for schizophrenia and bipolar disorder. However, individuals who carry a high burden of risk alleles for these disorders may display intermediate phenotypes which could have effects on fecundity or ability to find a partner; for example, genetic liability for autism has been associated with autistic-like traits in the general population and genetic liability for ADHD has been associated with impulsivity and lower educational attainment (Martin et al., 2014, Stergiakouli et al., 2017, Bralten et al., 2017). A body of research

on genetic correlations between traits has demonstrated positive genetic correlation between ADHD and extraversion and between autism, intelligence and educational attainment (Anttila et al., 2016, Sniekers et al., 2017). Genetic variants associated with educational attainment have previously been shown to be under negative selection in the Icelandic population (Kong et al., 2017).

No association was found between the PRS for schizophrenia and number of children, despite these being the most powerful PRS tested (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014, Mullins et al., 2017). However, such an association cannot be ruled out as there may be small genetic effects which the study was underpowered to detect. One advantage of using polygenic risk scores is that their predictive accuracy will improve over time and as larger discovery GWAS are conducted, other associations with fecundity may be uncovered. While this study found no evidence that schizophrenia risk alleles are under selection, a recent paper proposed a mechanism by which these variants are maintained (Pardiñas et al., 2016). Using data from the latest GWAS, common genetic variants for schizophrenia have been shown to be enriched in loss-of-function intolerant genes and to account for a substantial proportion of h^2_{SNP} (Pardiñas et al., 2016). Loss-of-function intolerant genes are by definition subject to strong selection pressure. When negative selection operates on areas of low recombination it can cause whole haplotypes to be removed from the gene pool, the result of which is that other haplotypes carrying mildly deleterious variants may increase in frequency by genetic drift (Pardiñas et al., 2016). Through this process of background selection, common variants for schizophrenia and other psychiatric disorders can be maintained, as long as they are not linked to a variant of large effect. Another recent paper investigated signatures of selection on 28 complex traits in the UK Biobank, by examining the relationship between risk allele frequency and effect size (Zeng et al., 2017). For a range of traits including height and body mass index, lower frequency variants tended to have larger effect sizes, indicating that these variants have been under negative selection, but again no evidence of selection was found for MDD-associated alleles.

Natural selection shapes the genetic landscape of heritable traits and studying this is now possible using population genetic biobanks, large-scale case-control samples and bioinformatics approaches for annotation. Investigating the selection pressures on genetic variants can illuminate the genetic architecture of psychiatric disorders and guide future gene-mapping studies. In turn, improved understanding of genetic architecture can fill the gaps in our knowledge about the mechanisms of natural selection on different types of genetic variation.

One final important finding from Chapter 4 is in relation to parental age. The link between advanced paternal age and risk of psychiatric disorders has widely been assumed to be due to *de novo* mutations in the spermatogonial stem cells of older males (Kong et al., 2012). Population genetic modelling has calculated that these mutations are unlikely to explain much of the paternal age effect, but a weak correlation between age at first child and genetic liability to psychiatric illness could account for the observed incidence of psychiatric disorders in the children of older fathers (Gratten et al., 2016). Chapter 4 advances the existing literature on paternal age by providing the first empirical evidence for this hypothesis. PRS for autism were associated with later age at first child in the Icelandic population (Mullins et al., 2017). Western society has experienced a rapid postponement of parenthood and if the paternal age effect is predominantly driven by inherited genetic variation, then it is unlikely that the prevalence of disorders such as autism and schizophrenia will increase, even as men have children later in life.

7.5 Genetics of suicidality

The last two chapters of the thesis focused on the genetics of suicidality, a serious and understudied comorbidity of psychiatric disorders with a well-established heritability. In Chapter 5, the largest GWAS on suicide attempt to date was reported, including over 6,000 attempters and 17,000 non-attempters with MDD, bipolar disorder and schizophrenia, recruited from the Psychiatric Genomics Consortium. The study found three genome-wide significant loci associated with suicide attempt in MDD or bipolar disorder implicating the *ARL5B* gene, an intergenic region between *IWS1* and *MYO7B* and a non-coding RNA *LOC105374524.* Replication of these associations in independent samples is essential and is part of a planned future collaboration, utilising samples of patients with mood disorders from the Danish population registry.

The sample size of this study is both its strength and limitation. It is the first consortium-based GWAS on suicide attempt, combining data across three psychiatric disorders and 46 constituent cohorts. The number of cases is five-fold larger than any previous GWAS on suicide attempt (Sokolowski et al., 2014). However, unsurprisingly, polygenic risk scoring and h^2_{SNP} calculations revealed that a further increase in sample size will be necessary to fully interrogate the common genetic architecture of this polygenic trait. In this GWAS, most support was seen for the chromosome 4 locus in *LOC105374524* and the association was specific to suicide attempt in mood disorders, rather than schizophrenia. There may be heterogeneity in the genetics of suicide attempt, for example between depressive versus psychotic attempts, but as polygenic

risk scoring analyses between datasets were underpowered, this is still unclear. As seen in GWAS of MDD, heterogeneity has profound implications for statistical power, so resolving the relationship between suicide attempt in different disorders is important for strategically planning future GWAS. An additional potential caveat is that one twin study estimated the heritability of suicide attempt after adjusting for psychiatric disorders to be 17% (Fu et al., 2002). This lower heritability would also reduce power, meaning that larger sample sizes will be required to detect genetic associations.

Prediction and prevention is imperative in reducing the burden of suicide. However, it is particularly challenging for this phenotype because clinicians often must rely on voluntary self-report from patients who are reluctant to disclose this information. There are currently no robust methods of assessing suicide risk and meta-analysis of 50 years of research on known risk factors such as psychiatric illness, demographics, family history and prior suicidal thoughts and behaviour, has shown that their predictive ability is weak (Franklin et al., 2017). Objectively measured biomarkers for suicidality are desirable but the field has been plagued by false positive results from small studies which do not replicate. In one such example, Le-Niculescu et al. (2013) reported that blood mRNA levels of *SAT1, PTEN, MAP3K3* and *MARCKS* are biomarkers of high versus low suicidality state and predictors of future hospitalisations for suicidality. Chapter 6 of the thesis conducted a direct replication of this study in the GENDEP antidepressant clinical trial and showed that the proposed biomarkers were not associated with suicidal ideation (Mullins et al., 2014a). This paper makes a valuable contribution to the scientific literature, given the plethora of limitations of the original study and the extensive press coverage of these biomarkers.

The advantage of blood gene expression biomarkers is that they are easily measured but also dynamic, which would be useful in monitoring patients during high-risk periods or for changes in suicidality during drug treatment. However, for a biomarker to be clinically useful it requires high sensitivity and specificity and like SNPs, gene expression biomarkers are likely to have small effect sizes which fall short of this threshold. We are still far from using biological tests to predict suicidality. Initial progress is being made using prediction algorithms developed from electronic health records and in the future combining biomarkers with other variables may be a good strategy to improve their predictive capacity (Smoller, 2017). For now, biomarker research can provide biological insights into suicidality, but studies need large sample sizes with robust phenotypes.

7.6 Future directions

There are several future directions to expand on the work presented in this thesis. Clearly, our understanding of the interplay between genes and environment is still fundamentally lacking and this is especially important for a disorder like MDD, which has a modest heritability and a strong environmental component. Studying genetic associations with psychiatric disorders has been accelerated through combining cohorts within consortia and the same could be possible for GxE research, with uniform or at least highly correlated environmental measures across cohorts. Naturally, an alternate approach is to conduct a single large homogenous GxE study. The UK Biobank is a prospective cohort study of ~500,000 individuals with genetic and rich phenotypic data from questionnaires and interviews, making it unprecedented in its size and scope (Sudlow et al., 2015). The resource has already been used to study GxE in the aetiology of body mass index and data from the mental health questionnaire on childhood abuse, traumatic events and current depressive symptoms will provide exciting future opportunities to dissect gene-environment interplay in depression (Tyrrell et al., 2017). Given the high genetic correlation observed between MDD and depressive symptoms, combining clinical and population level data in GxE research may also bring novel insights.

Further study of the association between genetic variants for psychiatric disorders and reproductive traits will be facilitated by continual improvements in the power of polygenic risk scores and the availability of population samples. It will be particularly important to follow up on the association between common risk variants and paternal age, given its implications for public health and searches for *de novo* mutations. In the future, increased coverage of the genome through whole genome sequencing will provide more insights into genetic architecture, allowing quantification of the contribution of rare variants and illuminating the selection pressures which have acted on the human genome.

For genetic studies on suicide attempt, identifying more associations will necessitate increased sample size. In the first instance, this could be feasible within the PGC, by extending this collaboration to working groups on other disorders where cohorts may have information available on suicide attempt, such as substance use disorders, eating disorders and post-traumatic stress disorder. The genetic relationships between suicidal ideation, suicide attempt and the psychiatric disorders themselves are currently not fully understood and could also be interrogated using genome-wide data. Even genetic associations with small effect sizes could yield invaluable insights into the biology of suicidality and bioinformatics methods are increasingly available for interpreting the molecular mechanisms underlying these associations.

Investments in genetic studies hold promise for the development of novel treatments which are much-needed to relieve the societal burden caused by suicide.

7.7 Conclusions

MDD is a heterogeneous disorder in symptoms, genetics and environmental risk factors. Genome-wide association studies have made progress at last in identifying the genetic variants involved and increases in sample size will lead to more discoveries. The availability of phenotypic, genetic and environmental data provides abundant opportunities to leverage the heterogeneity of depression in the quest to understand its complex aetiology. This thesis contributes to the scientific literature by dissecting the interplay between genetics and environmental risk factors, the selection on risk alleles at a population level and the genetic basis of suicide attempt, a serious symptom of MDD. Genetic studies of major depressive disorder provide promise for translating biology into new clinical tools for the treatment and prevention of this debilitating disorder.

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